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DIABETIC NEUROPATHY

Vibration Sense
and Abnormal Tendon Reflexes
in Diabetics

By
IB STEI

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ROM THE SECOND UNIVERSITY CLINIC OF INTERNAL MEDICINE
(Chief Professor K. Lundbæk, M.D.)
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Author's Previous Publications on This Subject

ratory Perception in Normal Subjects. A Biothesiometric Study
a med Scandinav 1957 *158* 315–325

ratory Perception in Diabetics. A Biothesiometric Study
a med Scandinav 1957 *158* 327–335

ratory Perception in Diabetics during Arrested Blood Flow to the Limb.
a med Scandinav 1959 *163* 195–205

ratory Perception in Non Diabetic Subjects during Ischaemia, with
cial Reference to the Conditions in Hyperglycaemia, after Carbohydrate
rvation and after Cortisone Administration.
a med Scandinav 1961 *169* 17–26.

uence of Diabetic Status on Vibratory Perception during Ischaemia.
a med. Scandinav 1961 *170* 319–338

Experientia fallax
judicium difficile

Preface

My studies on Vibratory Perception in Diabetics were commenced on the occasion of a prize essay on that subject set by the University of Aarhus. The essay was submitted anonymously marked "*Experentia fallax judicium difficile*" I feel that these words by Hippocrates are still valid.

When the studies were initiated, MOGENS MILFELDT M D allowed me to benefit from his experience in biothesiometric measurements.

Some of the results incorporated in the prize essay were published in the first two papers. These studies were made possible through my term as locum tenens at the First University Clinic of Internal Medicine, Aarhus Kommunehospital under the direction of Professor CAI HOLTEN M.D Both during this and my previous term as resident physician at the same clinic I learnt much from Professor HOLTEN's extensive knowledge and keen sense of criticism.

The studies on Vibratory Perception during Ischaemia were performed during my appointment at the Second University Clinic of Internal Medicine of Aarhus Kommunehospital where excellent working facilities were provided for me under the lively and inspiring direction of Professor KNUD LUNDBÆK, M D During the entire work I derived great benefit from Professor LUNDBÆK's wide knowledge in the field of diabetic research and his instructive help

During the various phases of my endeavours to contribute to the elucidation of neurological abnormalities in diabetics, I have met with great kindness and helpfulness from many of my colleagues among the medical staff of Aarhus Kommunehospital among these, I shall only mention POUL HONCKE, M.D., Head of the University Clinic of Physical Medicine and Rehabilitation and EJNER PEDERSEN M.D Chief Physician, the Department of Neurology

One of the prerequisites for the accomplishment of my studies has been the high quality of the daily work of the nurses of the hospital, who invariably met my special wishes with great compliance and readiness.

Many others have in various ways contributed essentially to the completion of the work presented in this monograph.

This report and the previous papers were translated from Danish into English by A. ROUSING, M T F who took great care in this task and also helped me in the solution of many practical problems during the printing of the book.

I offer my sincerest thanks to all those who have helped me.

Copenhagen, November 1961

IB STEINESS

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CHAPTER I

Diabetic Neuropathy

A Brief Survey

The occurrence of neurological abnormalities in patients with diabetes was well known in the last part of the nineteenth century at which time a large number of publications on the absence of tendon reflexes in the legs of diabetic patients appeared and neurological abnormalities presenting the picture of (poly)neuritis were also described. A survey of the early literature was given by AUCHÉ in 1890 With the exception of symptoms from the autonomic nervous system, the investigation of which was initiated by RUNDLES (1945) in his studies on orthostatic hypotension in patients with diabetes, little has been added to our knowledge of the clinical picture since the opening of this century

In the following review only a brief account of the numerous neurological abnormalities which have been described in, and ascribed to diabetes is given, as several detailed surveys are available (JORDAN 1936 RUNDLES 1945 BROCH & KLØVSTAD 1947 GOODMAN & AL. 1953 MARTIN 1953a, KEEN 1959 GARLAND 1960)

It is generally agreed that the neurological abnormalities in diabetes ar[illegible] most frequently localised to the extremities, above all, to the legs. It is partic[illegible] larly the sensory nervous system which is involved impairment of [illegible] function is rare when the effect of general weakness is disregarded.

The most frequent *subjective symptoms* are paraesthesiae and nocturnal [illegible] in the legs, usually of a cramplike character The paraesthesiae may [illegible] widely varying nature, e.g tingling or burning they may occasionally [illegible] accompanied by dysaesthesia or severe hyperaesthesia, so that even the [illegible] of the bedclothes is intolerable. However the subjective symptoms [illegible] erally mild in rare, severe cases, lancinating pain resembling that [illegible] may occur

Very often, it is difficult to assess the subjective symptoms, and [illegible] symptoms may also be encountered in non-diabetics. ALTHAUS ([illegible]

subsequent authors have emphasised the nocturnal character of the pain but although this is typical it cannot be designated as pathognomonic.

Mild or moderate subjective symptoms are relatively common. In my series (STEINESS 1957b) 13 (21 %) of 63 patients stated that they had often paraesthesiae during the period of examination supplementary information elicited from the patients or stated in the hospital records from previous admissions showed that a total of 20 patients (32) had experienced the occurrence of frequent paraesthesiae at some time 13 patients (21 %) reported that they had frequent episodes of nocturnal pain of a variable nature at some time. My series was relatively small but GOODMAN & AL. (1953) reported figures of the same order in a series of 261 patients 62 (24 %) had or had had paraesthesiae and 58 (22 %) had had pain at some time. Studies on the frequency of such symptoms in diabetics and a comparable control group are not available.

Severe subjective symptoms, sometimes accompanied by paresis, are rare. JORDAN (1936) found 25 patients of this category (neuritic type) among 1000 consecutive cases of diabetes within 30 months, but he made the reservation that the cause may not have been diabetes in all cases. On the other hand he believed that even in these the presence of diabetes did influence the course BROCH & KLOVSTAD (1947) encountered 19 (4 %) among 426 patients with diabetes, but likewise made some reservation concerning the diabetic aetiology

Owing to the uncertainty with which the evaluation of subjective symptoms in diabetics is beset some investigators, e g MATTHEWS (1955) and FAGERBERG (1959) did not accept these as a criterion for diabetic neuropathy In this connexion it is of interest to note that SKILLMAN & AL. (1961) observed a pronounced decrease in the nerve-conduction velocity in diabetics who complained of distressing subjective neuropathy with no objective neurological findings, while the nerve-conduction velocity was only slightly decreased in a group of patients who had neither subjective nor objective neuropathy This method of examination may perhaps open up a possibility of an objective evaluation of subjective neuropathy however this requires further investigation.

It is important to emphasise that subjective symptoms and objective signs are often dissociated. For example JORDAN (1936) described four types of diabetic neuritis. The first of these groups, the so-called hyperglycaemic type of neuritis, comprised patients with mild or moderate subjective symptoms in these cases, objective abnormalities were usually absent. Like JORDAN BROCH & KLOVSTAD (1947) divided diabetic polyneuritis into four types. Their first group comprised diabetics with subjective symptoms and no objective findings, and the fourth group consisted of patients with abolished

reflexes without subjective symptoms. In long-term diabetics, LUNDBÆK (1953) did not find any correlation between subjective symptoms and objective findings. In my own series (STEINESS 1957b) no correlation between subjective symptoms and objective findings could be demonstrated the subjective symptoms were not correlated to the duration of diabetes.

It is a well-known fact that the subjective symptoms are reversible, and it is generally agreed that they may occur at all stages of the disease—for example, frequently at the onset of diabetes. As distinct from the subjective symptoms, objective neurological signs, such as impaired vibratory perception on the legs and the absence of tendon reflexes in the legs, are significantly correlated with the duration of the disease as will be shown in the following chapters. From this it will be understood that subjective symptoms and objective neurological abnormalities in diabetics must be considered separately and that confusion may result if a differentiation is not made.

The most common *signs* are diminished or abolished tendon reflexes in the legs and impaired vibratory sense on the legs. Other types of sensory disturbances are rarer and often doubtful.

The *autonomic disturbances* comprise for example, diarrhoea, especially in the night orthostatic hypotension, bladder paralysis, sweat anomalies, pupillotonia—in very rare cases, Argyll Robertson pupil—and loss of potency These disturbances will not be discussed in the present study

In older patients with mild, but poorly controlled diabetes, asymmetrical, reversible paresis and atrophy of the extremital muscles, particularly of proximal localisation, have been described (GARLAND & TAVERNER 1953 SEITZ 1956 SKANSE & GYDELL 1956 SULLIVAN 1958, and BISCHOFF 1959 who has further references) This clinical picture, which has been termed *diabetic amyotrophy* (GARLAND 1955) develops relatively rapidly from a few weeks to months, and is often associated with pain in the affected region, while sensory disturbances are usually absent. Some of the patients have suffered from known diabetes for many years, whereas in others the disease has not been recognised but the presence of retinopathy has, in several of the cases, shown that the patients have actually suffered from subclinical diabetes for a relatively long period (SULLIVAN 1958 cf. LUNDBÆK 1955)

In the literature, diabetic amyotrophy is grouped under diabetic neuropathy " and SULLIVAN (1958) subdivided "diabetic neuropathy" into two forms the classic symmetrical distal neuropathy and an asymmetrical, predominantly motor neuropathy or radiculopathy " However it can hardly be regarded as definitively settled whether diabetic amyotrophy is, in fact,

due to a specific diabetic neurogenic affection. Clinically diabetic amyotrophy differs in all respects from the usual objective neurological manifestations in diabetics. Electromyographic studies (GARLAND 1955 SKANSE & GYDELL 1956, BISCHOFF 1959) have given rather uncertain results, often features suggesting a combination of neurogenic and muscular affection However non-paretic diabetics may also reveal electromyographic signs of neurogenic affection in the form of reduction in the number of motor unit action potentials and an increased proportion of polyphasic potentials (STEINESS 1958b, SKILLMAN & AL. 1961) Autopsy of two patients (SKANSE & GYDELL 1956, GARLAND 1960) did not reveal any significant changes in the spinal cord histological examination of the peripheral nerves in GARLAND's patient showed normal findings, whereas the muscles revealed striking changes." In biopsy specimens of muscular tissue BISCHOFF observed changes suggestive of a myodystrophic process, but not of any neurogenic affection.

Thus, the clinical picture is still rather obscure. However available data are in favour of a primary muscular affection rather than of a nerve lesion. In some cases, e g in patients with signs of pyramidal tract disease (GARLAND & TAVERNER 1953) vascular disturbances in the spinal cord may have been present. Thus, ELLENBERG & KRAINER (1959) described a patient in whom the clinical picture was very similar to that of so-called diabetic amyotrophy Six months after the onset of the disease the patient sustained acute coronary occlusion and died. Autopsy revealed a focus of incomplete malacia in the lumbar part of the spinal cord.

The classification of *diabetic artropathy osteopathy* and *trophic ulcers* is uncertain. The question is whether these conditions are due to neurological abnormalities or to vascular affection or perhaps a combination of both In this connexion it may be mentioned that AAGENÆS & HAAGENSEN (1959) in a biopsy specimen from a patient with diabetic artropathy found PAS-positive deposits in the vessel walls of the synovial membrane on the basis of this observation they suggested that the artropathy was due to a micro-angiopathy of the synovial membrane

In older diabetics, *ocular paralysis* of acute onset and less frequently *facial palsy* may sometimes be encountered. The frequencies reported vary to some extent VARBETH (1953) observed not less than five cases of ocular paralysis and two of facial palsy among 312 diabetic patients within a period of 29 months. As these paralyses do not differ clinically from similar paralyses in non-diabetics—they disappear spontaneously in the course of from weeks to months—there is no reason to believe that they should be referable to a specific diabetic abnormality Studies of control groups in order to reveal if these

TABLE 1

Statements by various authors as to the frequency of neurological abnormalities in diabetics. SEVRINGHAUS, who distinguished between subjective symptoms and objective findings, found no correlation between these his results are listed partly in Group I and partly in Group II.

Definition of diabetic neuropathy*	Authors	Criteria for diagnosis of diabetic neuropathy*	No. of patients	Neuropathy present	
				No.	%
Group I Objective findings	SEVRINGHAUS (1931)	Reduced reflexes.	500	–	46
	COLLENS & AL. (1946b)	Impaired vibratory sense	100	96	96
	BONKALO (1950)	Signs.	150	74	49
	MARTIN (1953)	Signs.	abt. 3000	150	5
	MATHEWS (1955)	Signs.	545	704	37
	FAGERBERG (1959)	Two signs	356	224	63
Group II Subj. sympt. and or objective findings	SEVRINGHAUS (1931)	Pains	500		36
	WENDT & PECK (1931)	Patients complaining incessantly and continually of pains.	1073	53	5
	RUNDLES (1945)	More permanent signs and symptoms.	abt. 3000	150	5
	BROCH & KLØVSTAD (1947)	Symptoms and/or signs	426	88	21
	AARSETH (1953)	Symptoms and or signs (apart from isolated loss of Ach. reflx. in elderly pts.)	312	21	7
	GOODMAN & AL. (1953)	Symptoms and, or signs	261	162	62
	HIRSON & AL. (1953)	Symptoms and or signs	100	57	57
	ROOT (in JOSLIN) (1959)	Symptoms and/or signs	3174	2061	65
Group III No definition	MURPHY & MOXON (1931)		827	5	0.6
	HAAGENSEN (1949)		188	4	2
	BECKMANN & AL. (1958)		1547	26	2

paralyses should be more frequent in diabetics than in non-diabetics have not been performed.

Several studies on the *spinal fluid* in diabetics with so-called diabetic neuropathy or polyneuritis have been performed (see, e.g., ROOT 1959) In these, it is reported that the protein content is slightly to moderately increased in 50–60 and markedly increased in about 10 On the other hand, pleocytosis is absent. It can hardly be excluded that some of these patients actually suffered from a genuine polyradiculitis. Studies of the spinal fluid in a comparable control group of diabetics without neurological abnormalities have not been performed.

TABLE 2.

Opinions expressed by various authors as to the significance of number of factors in the development of diabetic neuropathy

As regards the significance of the age factor it may be stated that it is generally agreed that diabetic neuropathy rarely if ever occurs in children, and is relatively rare in young individuals under 20 years of age

Authors	Age	Duration of diabetes	Control of diabetes	Severity of diabetes
WENDT & PECK (1931)	No	–	Yes	–
RUNDLES (1945)	No	No	Yes	No
BROCH & KLØVSTAD (1947)	Yes	Yes	N	No
AARSETH (1953)	Yes	No	–	Yes
GOODMAN & AL. (1953)	Yes	No	Yes	No
HIRSON & AL. (1953)	No	No	Yes	No
MARTIN (1953)	No	No	Yes	No
MATTHEWS (1955)	Yes	Yes	Yes	Yes
FAGERBERG (1959)	Yes	Yes	–	–

After this brief account of the most common neurological abnormalities described in the literature it may be reasonable to ask the question how often neurological abnormalities are encountered in diabetic patients. The statements in the literature as to this point vary within wide limits, among other things, because different criteria are used in the diagnosis of "diabetic neuropathy (Table 1) By this concept, some authors understand both subjective and objective neurological abnormalities of slight or severe degree. Others require the presence of severe subjective symptoms, and finally as already mentioned, during recent years there has been a tendency to restrict the term diabetic neuropathy" *to cases presenting objective signs of nerve lesion.* It is therefore not surprising that the frequencies reported show some discrepancies but largely they range from about 40 to 65 % both for the authors who only accept objective findings and for those who also include slight subjective symptoms. Severe subjective symptoms seem to occur only in a small percentage of the cases. As regards autonomic disturbances, it may be mentioned that ROOT 1959) observed so-called diabetic diarrhoea in 119 out of 3174 patients with diagnoses involving the nervous system seen during the period from 1946 to 1957

In conclusion it may be said that the picture of the neurological abnormalities in diabetics as it appears from the descriptions in the literature is somewhat obscure To this it must be added that the statements concerning the relation of the neurological abnormalities to the duration of the disease the

age of the patients, their insulin dose and the diabetic control are contradictory (Table 2) It should be noted that apart from a few studies on vibratory perception in diabetics, the series have not been checked for false correlations the results must therefore be taken with some reserve

As a contribution to the clarification of the occurrence of objective neurological abnormalities in diabetes, the results of studies of the vibratory sense in diabetics are discussed in Chapter 2.

For the purpose of comparison and substantiation of the results obtained by biothesiometric measurements an analysis was performed of abnormal tendon reflexes in the legs in my series of patients (STEINESS 1957b) and in other series in which the individual patients have been described in sufficient detail (Chapter 3)

Chapter 4 presents a conclusive discussion of the pathogenesis of the nerve affection in diabetics on the basis of the data described in this monograph and the findings in my studies on vibratory perception during ischaemia and other results of recent research.

Chapter 2

Vibratory Perception in Diabetics

Vibratory perception was previously studied by means of tuning forks, but during recent years it has become more and more common to use the so-called biothesiometers.

Tuning-fork examinations can be performed either qualitatively or quantitatively. In the qualitative examination the tuning fork is struck and placed on the site of measurement, and the patient is instructed to indicate whether or not he can perceive the vibrations. In the quantitative examination the time during which the patient can perceive the vibrations is measured by means of a stop watch. Some investigators provided the prongs of the tuning fork with special devices, so that it could be checked that the amplitude was of the same size at the commencement of the individual measurements.

A biothesiometer (Fig. 1) is an electromagnetic apparatus, by means of which a rod, or stylus, is brought into inaudible vibrations. In the most common type of apparatus, the amplitude is variable, while the frequency is constant, depending upon the frequency of the alternating current used. During the

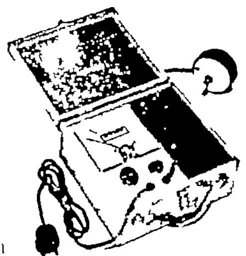


Fig. 1.—The biothesiometer used in the present study.

The vibrator with the stylus ending in bakelite cylinder with flat contacting surface, 12 mm in diameter is seen to the extreme right. The stylus vibrates with frequency twice that of the alternating current. The frequency of the AC current used was 50 cps. The amplitude is proportional to the square of the voltage applied. In the measurements, the stylus was placed at right angle to the skin, as far as possible resting with the whole weight of the vibrator 390 g. on the site of measurement.

The voltage is read directly on the scale and can be varied by the control knob to the left below the scale. The knob to the right is the main switch.

The biothesiometer is manufactured by the Bio-Medical Instrument Company, Chagrin Falls, Ohio.

measurement the stylus is placed at a right angle to the skin on the site of measurement, and the amplitude is gradually increased from zero by increasing the voltage The subject under test is instructed to report when he perceives the vibrations, at which time the amplitude or an arbitrary expression thereof, usually the voltage, is read directly on a scale. In this way a numerical expression is obtained for the vibratory perception threshold on the site of measurement for the subject under test.

Remarks on Studies of Vibratory Perception

Before the results obtained in studies of vibratory perception in diabetics are discussed it will be appropriate to consider a few important prerequisites for the evalution of the results. Other important factors have been described in a previous study (STEINESS 1957a)

TREITEL (1897) who was the first investigator that studied vibratory perception, found that this was best developed in the upper extremities that the sensitivity increased distally on the extremities and that it was poorly developed on the trunk. These observations have been confirmed by later investigators. Hands and feet are therefore suitable sites of measurements, whereas the results of biothesiometric measurements on the proximal parts of the legs are often questionable (cf. STEINESS 1958a)

EGGER (1899) observed that the vibratory acuity decreased with advancing age but the first study of the age factor in a large series was performed by PEARSON (1928) who by means of quantitative tuning-fork examinations revealed that the effect of the age factor is most pronounced on the legs, especially after the age of 50 years. PEARSON studied convalescents, but MIRFELDT (1957) confirmed his results in studies on healthy individuals, using tuning forks of different frequency Biothesiometric measurements have concordantly shown that the vibratory perception threshold shows a marked increase with advancing age on the feet but only a slight increase on the fingers (see also STEINESS 1957a)

Biothesiometric studies have revealed that the vibratory acuity varies within wide limits from individual to individual, especially on the feet and especially in elderly subjects. In persons of advanced age the vibratory perception threshold may therefore be relatively high without being an expression of significantly impaired sensibility Owing to the wide individual variations it is necessary to have a control series in order to assess the results in the patients.

The maximum sensitivity to vibratory stimulation is within the range from approximately 100 to 600 cps (GELDARD 1940) As early as 1897 TREITEL realised that the frequency might be of importance he found that the C tuning fork (frequency 128 cps) gave the best perception. However even for tuning

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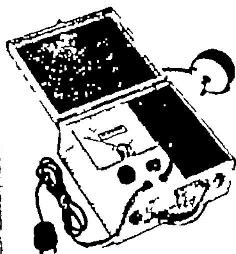


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It also appears from the diagram that identical percentual decreases in the vibratory perception in a young and an elderly person may very well lead to the situation that the vibratory perception threshold in the latter is increased just so much that he cannot perceive the vibrations of a casual large-sized tuning fork with an amplitude corresponding to that of the biothesiometer at, for example 30 or 40 volts, whereas the young individual in spite of impaired vibratory perception may distinctly feel these vibrations. In qualitative tuning fork examinations of young individuals, the risk exists that certain cases of impaired vibratory perception escape recognition.

Adaptation which as far as vibratory perception is concerned consists of a decrease in the sensibility during stimulation, constitutes an incontrollable factor in any quantitative tuning-fork examination, whereas it is avoided in biothesiometric measurements.

As far back as 1904 MINOR fully realised the fundamental disadvantages involved in tuning fork tests, but the understanding of these disadvantages gained ground only very slowly MINOR suggested that electrically activated tuning forks should be employed, and such tuning forks were used in diabetics as late as by COLLENS & AL. (1946a) However owing to the advent of the superior biothesiometers, electrically activated tuning forks have not been widely used

The application of biothesiometric measurements marked a great advance in the technique of neurological examination. For the first time, it now became possible to determine the threshold for an ordinary sensory impression with sufficient rapidity ease and accuracy which, in turn, opened a possibility of a more exact study of a neurological abnormality in diabetics. However before the biothesiometric measurements in diabetics are considered, it will be reasonable to describe the results obtained by tuning-fork tests.

Tuning-Fork Tests in Diabetics

The first study on vibratory perception in diabetics was published by WILLIAMSON (1905) who reported that the vibratory sense was often lost or greatly impaired in these patients.

WILLIAMSON employed an ordinary tuning fork— a large one, about six inches long"—and studied vibratory perception qualitatively on the styloid process of the ulna, the medial malleolus and on the inner surface of the tibia at about the middle of the bone. In a control group consisting of 50 healthy men, the vibrations were felt distinctly on both sides in every case over the three regions. The same observations were made in a group of 75 patients with various medical diseases, but in 15 out of 45 diabetics vibratory perception on the legs was lost or greatly impaired, and two of these patients also failed

to perceive the vibrations over the ulna. On the basis of these observations, WILLIAMSON concluded that with the technique used vibratory sense is always present under normal conditions, but is impaired in diabetics. All the patients with impaired vibratory perception were over 40 years old and had mild diabetes. Incidentally WILLIAMSON did not give any information as to the age *of the other patients or the controls, or as to the duration of diabetes, etc.* However it must be remembered that this study was performed long before the discovery of insulin at a time when juvenile diabetics survived for only short periods.

In 1922 WILLIAMSON summed up his experiences gained during 17 years study of vibratory perception in diabetics. He emphasised that he had invariably used the same large tuning fork and found that vibratory perception on the legs was often lost in diabetics, even though other forms of sensation were felt. In these diabetics, the Achilles-tendon reflexes and, less frequently the patellar reflexes were often absent. This observation is very interesting as it provided evidence in favour of the assumption that the neurological lesions which are responsible for these two abnormalities are of the same nature.

Apart from this, the literature on tuning fork tests of vibratory perception in diabetics is very sparse, and detailed investigations in large groups of patients and control series with analysis of the results in relation to the age of the patients and the duration of diabetes have not been performed With one exception (BARACH) the reports available are either incorporated in papers by *investigators who studied vibratory abnormalities in various types* of neurological patients, or in papers on neurological abnormalities in diabetics in general.

By means of quantitative tuning-fork tests, SYMNS (1917) confirmed WILLIAMSON's findings 12 patients with diabetic neuritis all revealed impaired vibratory perception on the legs, whereas only diabetics with neuritic involvement of the arms also showed deficient vibratory sensation on the upper extremities. SYMNS's control group consisted of 30 normal subjects aged from 18 to 30 years. No information was given as to the age of the diabetics the four patients whose ages are stated on the diagrams were all over 30 years. No mention was made of the duration of diabetes.

In a paper on quantitative variations in vibratory perception, WOOD (1922) laconically stated that vibratory perception improved in diabetics given proper treatment. No further comments are given on this interesting observation.

AHRENS (1925) reported that in diabetic patients with neurological findings vibratory sensation usually showed the same generalised impairment as is found in any other case of peripheral neuritis.

GORDON (1936) studied vibratory sensation in 25 diabetics by the same

technique as had been used by SYMES. No mention is made of the composition of the group. Ten of the patients had absent or deficient vibratory sensation on the lateral malleolus, but there was only one who showed loss of perception on the ulna. In addition, GORDON revealed impaired or abolished sensation on the sternum in five and over the sacrum in 14 patients. In biothesiometric measurements performed in 24 diabetics, COSH (1953) later found a normal threshold of vibratory sense on the sternum and over the sacrum in all cases. This discrepancy may obviously be due to a number of factors, but as already mentioned, vibratory perception is poor on the trunk, and the great individual variations in the amount of subcutaneous fat also contribute to making these regions unsuited for measurements.

BARACH (1947, 1949) performed tuning-fork tests in 150 diabetics over a number of years, in some cases for more than 15 years. He found that this form of examination was helpful in evaluating the condition of the patients and the progress of the disease, and wrote that it was a striking observation to see the curve approach normal or recede away from normal with the improvement or aggravation of the diabetic state. In this connexion it should be mentioned that I did not find any correlation between the vibratory perception threshold and the immediate diabetic state in biothesiometric measurements. In 12 patients in whom the diabetic state was varied within wide limits with a view to determination of the vibratory sensibility during ischaemia (STEINESS 1961 b) I measured the vibratory perception threshold on the index fingers and great toes before the measurement performed during arrested blood flow. The technique used failed to reveal any significant variations in the threshold when the diabetic status was changed in this way. The small differences from time to time did not show any tendency to impaired sensibility in the presence of a poor diabetic status.

In many papers on diabetic neuropathy cursory information is given of impaired vibratory sense without any accompanying statement as to the technique of examination. In a study on 125 patients with "diabetic neuropathy" RUNDLES (1945) found that vibratory sense was diminished in 37 and absent in 20 patients. Similarly MARTIN (1953 a) observed loss of vibratory sensation in two thirds of 150 diabetics with "diabetic neuropathy." He did not state the sites used for the measurements. Such information is of little value.

Loss or abolition of vibratory sensation determined by qualitative tuning fork tests has been used as a diagnostic aid in the disclosure of diabetic neuropathy (e.g. MATTHEWS 1955, FAGERBERG 1959). As will have appeared from the brief fundamental considerations here, this criterion must be taken with some reserve.

In conclusion it may be said that tuning-fork tests for vibratory sensation

in diabetics have shown that this sensation is often diminished or lost on the legs, but only in exceptional cases on the arms. According to WILLIAMSON's observations, impaired vibratory perception is often associated with loss of the Achilles-tendon reflexes. However tuning-fork tests are beset with important fundamental sources of error and these objections may be made against all available studies

In studies in which quantitative tuning-fork tests have been employed a correlation between the diabetic control and the vibratory sensation has been reported. *As already pointed out my own biothesiometric measurements of* the vibratory perception threshold failed to reveal any changes referable to variations in the diabetic status. If the results of tuning fork tests are reproducible, it may be possible that the changes observed are due to alterations in the adaptation in diabetics.

Biothesiometric Measurements in Diabetics

As previously pointed out, biothesiometric measurements offer the immediate fundamental advantage that it gives a direct numerical expression of the threshold for the sensory impression which is studied It will be reasonable to include the investigations by COLLENS & AL. in which they used an electrically activated tuning fork in this section

By biothesiometric measurements it has not only been possible to confirm the principal conclusions drawn from the tuning fork tests but also to extend our knowledge of the impairments of vibratory perception.

The biothesiometric studies are discussed and compared below in order to clarify the divergences existing between the various studies

COLLENS & AL. performed a series of studies on vibratory perception in large groups of diabetics. They used an electrically activated tuning fork (COLLENS & AL. 1946a) and measured the threshold on index fingers and great toes.

These investigators found very frequently impairment in the vibratory sense in diabetics. Of 100 patients with peripheral neuritis, 90 % had diminished vibratory sense on the upper extremities and 98 % on the lower extremities in 100 diabetics without peripheral neuritis, the corresponding figures were 74 and 96 % (COLLENS & AL. 1946b) Both in the presence and absence of peripheral neuritis the vibratory sensibility returned to normal or improved in more than one half of the cases as a result of intensive treatment with vitamin B complex this improvement began within a few weeks (COLLENS & AL. 1946c, 1950a) In a study of 100 diabetics with and 100 without proteinuria (COLLENS & AL. 1950b) they found that the former had a much more profound impairment in vibration sense than the latter In all their studies,

it is emphasised that impaired vibration sense was not related to the duration, severity or degree of control of the disease nor was any correlation with the duration of diabetes revealed in the groups with and without proteinuria.

The high frequency of impaired vibration sensation in these studies is incompatible with the results of biothesiometric measurements, which have shown a wide overlapping of the threshold values obtained in diabetics and non-diabetics (see e.g., the diagrams in Mirsky & al. 1953 Steiness 1957b, and Jersild & Lauritzen 1957) A possible cause of this discrepancy is that the normal range had been made too narrow Collens & al. measured the threshold in arbitrary units and the starting point was the minimum amplitude of vibration which could just be detected by the normal subjects. This minimum threshold was determined in studies on 100 normal, non-selected individuals (Collens & al. 1946b) but no details are given, and the ages of the control subjects are not stated. If the control subjects have largely belonged to the younger age groups and the diabetics been relatively old this will result in too large a number of abnormal threshold values. This assumption is supported by the fact that Collens & al. found a considerably lower percentage of abnormal values among 50 young patients, viz. 43 % on the legs and 14 on the arms.

However the objection raised cannot explain the observed effect of the treatment with vitamin-B complex, which was quite independent of the diabetic control At the present time when the hypothesis of vitamin B deficiency as a pathogenic factor in diabetic neurogenic affection must be regarded as obsolete it is difficult to explain this observation which has not been further studied by other investigators.

Cosh (1953) studied the threshold of vibration in the fingers and toes of 24 diabetic patients, most of whom were undergoing stabilisation in hospital. Threshold were measured under standard conditions with a biothesiometer and the results were compared with the findings in a control series. As the upper limit of normal was used the average threshold obtained in the control series for the patient's decade plus twice the standard deviation. The control group consisted of only five individuals for each decade of life.

It appears from the study that the patients who had the severest neurological symptoms and signs also revealed the most severely impaired vibratory perception. These patients had the longest average duration of diabetes and the highest average age, but as allowance was made for the influence of age in the evaluation of the threshold, the age factor may be disregarded. There was very good agreement between the impairment of vibratory sensation and the other neurological symptoms and signs, but in the evaluation of the results it must be remembered that the series was relatively small.

MIRSKY & AL. (1953) studied vibratory perception on the index fingers and great toes of 102 diabetics and 136 non-diabetics in the age groups from about 5 to about 80 years. The determinations were made by means of a biothesiometer under standard conditions. The results of the measurements were subjected to a detailed statistical analysis in which allowance was made for the age factor MIRSKY & AL. were the first to study if there was any difference in the vibratory perception in men and women

The most important results of their study were as follows —

(a) In the diabetics, the vibratory perception threshold was significantly increased both on the fingers and toes. At both sites of measurement the threshold increased significantly with age uniformly in diabetics and non-diabetics.

(b) In non-diabetics, MIRSKY & AL. observed a barely significant sex difference on the great toe the threshold being higher in men than in women. On the right great toe this sex difference was significant at the 5 % level on the left at the 1 % level

(c) By a multiple regression analysis with three variables—age duration and severity (as expressed by the insulin dose) of diabetes—it was not possible to demonstrate any correlation between the increase in threshold and the duration or severity of diabetes.

STEINESS (1957a, b) studied vibratory perception in 100 non-diabetics and 63 diabetics under 60 years of age The threshold was measured bilaterally by means of a biothesiometer under standard conditions on the index finger over the styloid process of the radius over the medial malleolus and on the great toe.

The most important results of this study were as follows —

(A) The findings mentioned under point (a) in MIRSKY & AL. were confirmed.

(B) It could be clearly demonstrated that the threshold on the great toes was higher in men than in women among non-diabetics. This was also the case on the medial malleolus, although the difference was less pronounced. On the other hand diabetics did not reveal any sex difference.

(C) On the great toes, a statistically significant correlation was demonstrated between vibratory threshold and duration of diabetes (Fig 3 A) but the study failed to reveal any correlation with the severity of the disease (as expressed by the insulin requirements) or with the subjective neurological symptoms, which were however very mild in all cases. Nor could any correlation with the estimated control of diabetes be demonstrated, but the composition and size of the series did not allow definite conclusions as to this point.

On the index fingers, no correlations could be demonstrated this applies especially to the duration of the disease (Fig 4 A)

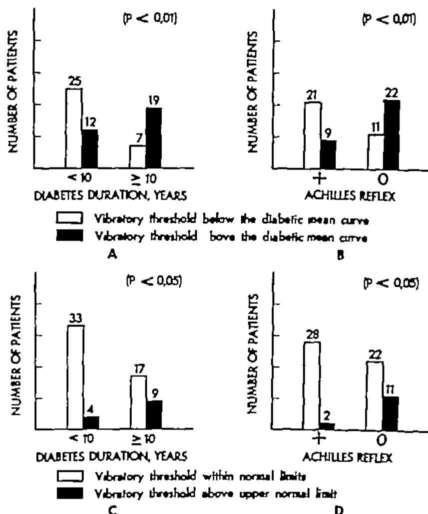


FIG 3 —The vibratory perception threshold on the great toe related to the duration of diabetes and the occurrence of abnormal reflexes in the legs.

The figures bove the columns indicate the numbers of patients.

The P alues were calculated by χ^2 test.

(D) On the great toes, high vibratory perception threshold was significantly correlated with other objective signs of neurogenic lesions, i.e. abolished tendon reflexes and impaired sensibility for touch, pain and temperature.

On the index fingers, no correlations could be demonstrated.

Supplementary Analyses of Own Series

In addition to the vibratory perception, special attention was focused on

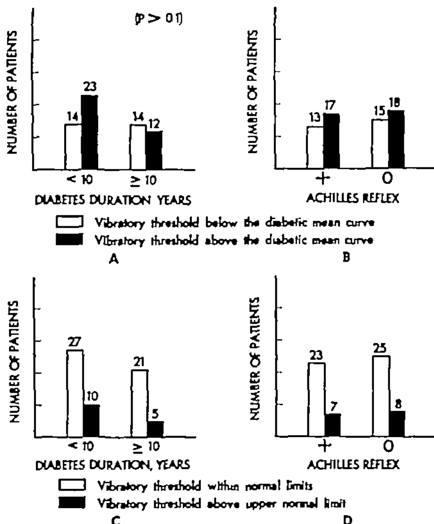


FIG. 4.—The vibratory perception threshold on the index finger related to the duration and the occurrence of abnormal reflexes in the legs.
The figures above the columns indicate the numbers of patients.
The P value was calculated by χ^2 test.

loss of tendon reflexes in the legs as a manifestation of neurological affection in the patients (see also Chapter 3)

Among the patients who in the previous study (STEINESS 1957b) were included in the group of "objective neuropathy" there were, however four in whom the reflexes were *not* lost, but who were described as suffering from "objective neuropathy" because sensory disturbances were believed to be present. The correlation analyses which had previously been performed were therefore supplemented by a special study of the patients with loss of re

flexes. As the Achilles-tendon reflexes are the first to be lost in diabetics (Chapter 3) loss of reflexes does in this connexion mean loss of the Achilles-tendon reflexes.

Principle of analysis —The analyses were performed according to the same principle as was used in the previous correlation analyses (STEINESS 1957b)

(i) It was first studied how many of the patients with abnormal reflexed had a treshold higher than the calculated mean curve for diabetics This distribution was then compared with that of the patients without areflexia. The patients with threshold values below and above the curve for the mean values were characterised as having a low and a high vibratory perception threshold respectively

(ii) In the present study an analogous analysis of the distribution of the threshold values as related to the calculated upper limit for the normal variations (upper normal limit) was performed. The threshold values below this limit were characterised as normal those above as increased." In this connexion it must be emphasised that a threshold value within the range of normal variations may very well be relatively increased if the patient, for example before the onset of diabetes, has had a low threshold and now has a value just below the upper normal limit. Conversely an increased value may represent a normal variant which falls outside the calculated range of normal variations.

Theoretically the upper normal limit should correspond to the 97.5 % level As the transformation function as described did not completely correct the skewness (cf. STEINESS 1957b) and as the limits were calculated from a limited series, they must be taken with some reserve. As might be expected the curves did not completely fulfil the ideal requirements. Thus, of the values for the normal group 4 % for the index finger and 6 % for the great toe were above the normal limit, as against the theoretically excepted 2.5 % If, however the aforementioned factors of uncertainty are taken into account, the curves agree fairly well with the actual conditions (Fig 2 page 24)

Results

(i) For the great toe, there was a distinct correlation between high vibratory perception threshold and loss of the Achilles-tendon reflex (Fig 3 B) the χ test showed that this difference was statistically significant ($P < 0.01$)

For the index finger no correlation could be demonstrated (Fig 4 B)

Conclusion —The results of these analyses of high" and "low" vibratory perception threshold values in relation to abnormal reflexes are indentical with the observations made in the previous studies as regards the patients included in the group of objective neuropathy

(ii) With regard to "increased" vibratory perception threshold the distribution for the great toe also suggests that a correlation exists between impaired vibratory sensation and loss of the Achilles-tendon reflexes (Fig 3 D): there were only two patients with normal Achilles-tendon reflexes in whom the vibratory threshold was "increased." A χ^2 test with Yates's correction revealed that the difference was significant ($P < 0.05$)

As distinct from this, there was no correlation between "increased" vibratory threshold on the index finger and loss of Achilles-tendon reflexes (Fig 4 D)

Conclusion.—The results of the analyses as regards "increased" vibratory threshold were identical with those obtained when the curve for the calculated diabetic mean values was used in the analysis.

Comments on the correlation analyses.—As previously mentioned this series revealed a statistically significant correlation between long term diabetes and a "high" vibratory perception threshold on the great toe (Fig 3 A): a similar correlation was also found as regards the upper normal limit (Fig 3 C) As appears from Chapter 3 the occurrence of abnormal reflexes in this series was also correlated with the duration of diabetes. Accordingly these analyses provide evidence in favour of the assumption of a common pathogenic factor as the cause of these two manifestations of nerve lesion.

JERSILD & LAURITZEN (1957) studied vibratory perception in 188 non-diabetics aged from 5 to 85 years and in 288 diabetic patients aged from 5 to 80 years. Their results were not subjected to statistical analysis, but their illustrations give a good impression of the observations made In the diabetic group the threshold on the index finger was distinctly increased and the dispersion was wider than in the normal group. This was also the case for the great toe, and here the threshold was higher in the group with long term diabetes than in the group with diabetes of short duration, i e. less than 10 years. For patients with either subjective or objective neuropathy the threshold was increased as compared with the remaining diabetics the average duration of diabetes in these two groups was not stated but it is seen from their Figure 11 that neuropathy was more frequent in patients with long term diabetes than in those who had suffered from the disease for less than 10 years. The patients with retinopathy had a distinctly higher threshold on the great toe than the remaining patients this finding may suggest a correlation between angiopathy and nerve lesion.

DISCUSSION OF THE BIOTHESIOMETRIC MEASUREMENTS

Tuning-fork tests showed that vibratory sensation is often impaired in diabetics, at least on the legs. However in view of the fundamental objec

tions which may be made against these tests and the less detailed elaboration of the results obtained it is hardly justified to draw any further conclusion.

On the other hand, the reports on biothesiometric measurements provide a weighty material for the clarification of the abnormal vibratory sensation in diabetics. As appears from the preceding review good agreement exists on many points, whereas essential details disagree, and it is difficult to reconcile all information available. Below the principal features will be outlined, and the discrepancies are discussed in the light of the information available.

It may be regarded as well established that the mean vibratory perception threshold in a group of diabetics is increased in comparison with a corresponding group of non-diabetics, and this applies to both hands and feet. However the values in the two groups show a distinct overlapping. These findings clearly appear from the diagrams published in the papers of MIRSKY & AL., STEINESS and JERSILD & LAURITZEN and are statistically verified in the first two of these studies. The diagrams also show that the dispersion is greater in the diabetic groups than in the control groups.

In diabetics as well as in non-diabetics, the threshold increases significantly with age, both on the toes and fingers (MIRSKY & AL., STEINESS); this increase with advancing age also appears from the diagrams in the paper published by JERSILD & LAURITZEN.

By the statistical analysis MIRSKY & AL. found a "barely" significant sex difference in non-diabetics, the vibratory perception threshold on the great toe being higher in men than in women. In my series, this sex difference was clearly significant. In view of these findings, it must be regarded as a reality. This difference is of interest in comparison with the frequency of the manifestations of diabetic vascular lesions, in which the sex ratio is 1:1 as distinct from the arteriosclerosis of the vessels of the heart and lower extremities in non-diabetics, which is most frequent in men.

As MIRSKY & AL. could not demonstrate any correlation between the increased vibratory threshold in diabetics and the duration or severity of the metabolic syndrome, they concluded that the impairment in vibratory perception must be considered to be a "concomitant" to diabetic disease.

As distinct from the findings by MIRSKY & AL. my series revealed a statistically significant correlation between the duration of diabetes and a high vibratory perception threshold on the great toe. Evidence in support of this statistically demonstrated correlation is provided by the fact that six patients in the series were unable to feel the vibrations on the great toe at maximum amplitude; in all six cases, the diabetes was of long duration (average 19.3 years, range 10–32 years). The diagram in JERSILD & LAURITZEN's paper distinctly reveals a correlation between the duration of diabetes and the impairment in vibratory perception on the great toe, but this finding was not

statistically verified This relation is also supported by the studies of COSH. There is thus strong evidence in favour of the assumption that the demonstrated correlation with the duration of diabetes is a reality

In the clarification of the objective neurological abnormalities in diabetics it is important to decide whether the impairment in vibratory perception progresses with the duration of diabetes. Apart from the studies of COLLENS & AL. only the findings by MIRSKY & AL. speak against this assumption. On the other hand, the series studied by MIRSKY & AL. is so large and was so carefully analysed that appreciable weight must be attributed to the results obtained.

As MIRSKY & AL. and I studied the patients in the same way under standard conditions, it is most likely that the discrepancy is referable to differences in the composition of the two series.

In both series, patients with other diseases which are known or must be assumed to impair vibratory perception were excluded. However in contrast to MIRSKY & AL. I did not exclude patients with symptoms of vascular insufficiency in the legs. As previously reported (STEINESS 1957b) seven of the patients in my series had such symptoms. As will be mentioned later there is, however no evidence suggesting that demonstrable arterial insufficiency is of any direct pathogenic importance in neurogenic affections.

As both series were corrected for the age factor this can be disregarded. On the other hand my series comprised a larger proportion of patients with long-term diabetes than that considered by MIRSKY & AL. In my series, 26 (41 %) of the 63 patients had had diabetes for 10 years or more, while judging from the diagrams in the paper of MIRSKY & AL. this was the case with only 23 (23 %) of their 102 patients. It is therefore possible that the "concomitant" factor may have exerted so strong an influence in the statistical analysis that it has blurred a possible superimposed factor correlated with the duration. However a study of their diagram does not reveal any tendency to a higher threshold in patients with long term diabetes.

As previously pointed out, my series revealed a statistically significant correlation between the ordinary objective signs of neuropathy and the duration of diabetes. It is therefore of importance that MIRSKY in another paper (1953) reported that a series of 102 diabetics (which must be supposed to be identical with that studied biothesiometrically) did not reveal any significant association between the frequency of ordinary objective signs of neuropathy and the known duration of the metabolic syndrome. Our two series therefore seem to differ from a neurological point of view and are thus not comparable The question is then Which of the two series should be considered to be representative of a population of diabetics? This question will be discussed in Chapter 3

In conclusion it may be said that biothesiometric measurements have concordantly shown that the vibratory perception threshold is increased in diabetics.

The impairment of the vibratory sensation seems to comprise two components —

(1) A "concomitant" threshold increase, i.e. one unrelated to the known duration of the metabolic abnormality. The results of the studies by MIRSKY & AL. and the fact that my studies did not reveal any correlation with the increased vibratory threshold on the index fingers are in favour of this assumption.

(2) A superimposed threshold increase, which is correlated with the duration of diabetes. This correlation between the duration of diabetes and the impairment of vibratory perception has with great probability been demonstrated on the legs, but not on the arms.

In order to elucidate whether a nerve lesion develops with increasing duration of diabetes, the frequency of abnormal reflexes in diabetics will be discussed in Chapter 3.

CHAPTER 3

Abnormal Reflexes in Diabetics

In the preceding chapter it was discussed whether or not impairment in vibratory sensation increases with the duration of diabetes. In a series which was subjected to detailed statistical analysis, MIRSKY & AL. (1953) failed to demonstrate any such correlation, while my own series revealed a statistically significant positive correlation. Other biothesiometric studies are in support of its correlation with the duration of diabetes, but as no statistical evaluation has been performed, the problem cannot be regarded as definitely clarified.

In my series there was also a significant positive correlation between the duration of diabetes and other objective signs of neurogenic affection while this was not the case in the series studied by MIRSKY & AL. (MIRSKY 1953) The two series thus differ with regard to neurological findings, and it is reasonable to explain the discrepancy as regards the impairment in vibratory perception on this basis.

The purpose of the present chapter is to discuss which of the two series can, from a neurological point of view be regarded as representative of a population of diabetics, and hence as reflecting the actual conditions in diabetics. This coincides with the problem whether the occurrence of objective neurological abnormalities in patients with diabetes is correlated with the duration of the disease

In order to throw light on this problem, I decided to study the occurrence of abnormal reflexes in the legs in my series and to compare the findings with the data available in the literature. The reasons why the investigation was limited to comprise only a single manifestation of neurogenic affection were —

(a) Loss of the tendon reflexes in the legs is generally recognised as an early manifestation of neurogenic affection in diabetics

(b) Examination of the tendon reflexes in the legs is so simple that good agreement between the findings of various authors must be expected As distinct from abnormal reflexes, disturbances of sensibility in diabetics are most frequently very mild and the results of the latter type of examination are therefore beset with a considerable amount of uncertainty

Literature on Tendon Reflexes in Diabetics

BEFORE THE ADVENT OF INSULIN

In 1880's several investigators found, independently of each other, that the patellar reflexes were often absent in diabetics (MARINIAN (Italy) 1884, BOUCHARD (France) 1884, ROSENSTEIN (Holland) 1885, RAVEN (England) 1887). The first study on loss of the Achilles tendon reflexes was published by WILLIAMSON (1903), who found that the tendo Achillis jerk is the deep reflex which is first and most frequently lost. In 1924 WILLIAMSON reported that the tendon reflexes in the arms are always present.

The reason why attention was for such a long time exclusively focused on the patellar reflexes is undoubtedly that WESTPHAL in 1875 had reported that the patellar reflexes are absent in tabes. At the time concerned, it was therefore a very important observation that loss of the patellar reflexes might occur in diabetics not suffering from tabes.

Among the first reports, the series considered by BOUCHARD was by far the largest. During a period of three years, BOUCHARD found loss of reflexes in 19 of 66 diabetics, and only in exceptional cases was the areflexia reversible. A survey of the early literature was published by NIVIÈRE in 1888.

The occurrence of loss of patellar reflexes was rapidly confirmed by many investigators, and in 1892 EICHHORST stated that this observation was a generally recognised and well-established clinical fact.

The statements as to the frequency of loss of the patellar reflexes varied to some extent in the various reports (Table 3). Owing to the great difference between his own findings and those reported by GRUBE, WILLIAMSON (1897) made a detailed analysis in order to reveal the cause of this discrepancy. His conclusion was that the difference was apparently due to the fact that his own patients were relatively young, mostly suffering from a very severe form of the disease and often in a very advanced condition, while GRUBE's patients were older and suffered from milder diabetes.

TABLE 3

The occurrence of doubtful or abolished patellar reflexes in diabetic patients in old large series.

Authors	No. of patients	Abnormal patellar reflexes	
		No.	%
BOUCHARD (1884)	66	19	29
MASCHKA (1883)	36	18	50
EICHHORST (1892)	48	9	19
GRUBE (1893)	131	10	8
WILLIAMSON (1897)	100	49	49

Even though the reports from the pre-insulin era do not allow any detailed interpretation of the importance of the factors which might directly be assumed to influence the development of abnormal reflexes, they do contain observations which are still of interest.

Among 100 patients (39 under and 61 over 30 years) WILLIAMSON (1897) found loss of patellar reflexes in 30 of the former and in 25 of the latter group. In a smaller series of 39 patients, GRUBE (1895) had found a somewhat higher incidence of loss of reflexes in patients over 50 years, but his series did not allow definite conclusions as to the influence of age. Among 450 patients with diabetes, KRAUS (1922) observed abnormal patellar reflexes in 40 %. Figure 1 in KRAUS's paper does not show any tendency to correlation between abnormal reflexes and age in his series. From these reports it can be concluded that the patellar reflexes may apparently be lost both in younger and older individuals, and both in mild and severe cases of diabetes.

ROSENSTEIN (1885) did not find any correlation between loss of patellar reflexes on one hand and the severity of glycosuria or acetonuria or the general condition of the patient on the other. Many single reports on the return of tendon reflexes in patients whose diabetic state improved during treatment are available, but systematic studies aiming at the documentation of such reversibility are missing. In agreement with the statements of BOUCHARD (1884) GRUBE (1895) who had great personal experience, stated that this alleged reversibility was at variance with his observations. GRAM (1924) reported that the patellar reflexes are lost during coma as contrasted with this, it may be mentioned that many earlier investigators had expressed surprise at the observation that these reflexes may be present in coma, right up to the occurrence of death (GRUBE 1893 1895 WILLIAMSON 1897 NAUNYN 1906) The presence of reflexes in deep coma has been taken as evidence in favour of the assumption that loss of reflexes cannot be due to the metabolic abnormality per se. In this connexion it may also be mentioned that WILLIAMSON (1897) emphasised that the patellar reflexes are usually present in the earliest stage of diabetes, even in its most severe form, and that they only disappear during the progress of the disease.

AFTER THE ADVENT OF INSULIN

Frequency of Abnormal Reflexes

The introduction of insulin into the therapy of diabetes (1922) all at once changed the picture of the population of diabetics. Young diabetics were no longer mortally ill, but survived with the disease in well-being. Thus, the findings from before and after the introduction of insulin treatment cannot be compared straightaway

In the optimism which prevailed in the years just after the advent of insulin it was supposed that abnormal reflexes in diabetics would now disappear Thus, WOLTMAN & WILDER (1929) wrote "Before insulin became available areflexia was frequently encountered in juvenile patients. In all the juvenile cases reflexes returned with the return of the normal state of nutrition after insulin. However this categorical assertion was not documented.

WOLTMAN & WILDER's assertion was supported by MELANDER (1931) who by a review of hospital records studied the frequency of abnormal re

TABLE 4

Frequency of abnormal tendon reflexes in diabetic patients in more recent large series.

Authors	No. of patients	Absent patellar reflexes		Absent Achilles-tendon reflexes		Absent "tendon reflexes"		Comments
		No.	%	No.	%	No.	%	
SEVRINGHAUS (1931)	500		46					Diminished reflexes.
BROCH & KLOVSTAD (1947)	424	45	11	76	18			
BONKALO (1950)	150					74	49	
AASETH (1953)	506			93	30			Absent Achilles-tendon reflexes or greatly diminished tendon reflexes.
GOODMAN & AL. 1953)	261	67	27	126	48			
HIRSON & AL. (1953	100	19	19	63	63			
KARDEVO (1956)	270						26	Tendon reflexes diminished in 14.4% and abolished in 11.5%
FAGERBERG 1959)	338	140	41	210	62			
STEINESS (1957b)	63	15	24	33	52			Absent reflexes in series studied by biothesiometry
SKILLMAN & AL. (1961)	103		22		44			According to Fig. 2.
Long-term diabetes								
CHUTE (1948)	27					9	33	Absent patellar or Achilles-tendon reflexes. Duration of diabetes more than 15 years.
LUNDBÆK (1953)	157	40	25	68	43			Absent or doubtful reflexes. Duration of diabetes 15–25 years.
COWTAN & AL. (1954)	103					27	26	Loss of at least two tendon reflexes in the legs. Duration of diabetes more than 20 years.

flexes in diabetics in Gothenburg during the first seven years after the introduction of insulin. According to his analysis, the frequency of abnormal reflexes ranged from less than one to a few per cent., and MELANDER concluded that this is hardly greater than the habitual occurrence. However these figures have not been supported by other observers. In a similar series from the U S A., SEVRINGHAUS (1931) reported that reduced patellar reflexes were observed in 46%

The frequencies of abnormal reflexes reported in various studies vary to some extent, just as was the case in earlier reports, but they are of the same order of magnitude. JORDAN (1936) stated that more than 40% of all diabetics showed sluggishness or absence of tendon reflexes of the legs. The frequencies of abnormal reflexes observed in some series which have been published after the introduction of insulin are listed in Table 4 It must be concluded that the incidence of abnormal reflexes in diabetics is at least just as high as before the advent of insulin.

Just as in the older literature, occasional reports on cases in which return of the tendon reflexes in diabetics with severe "diabetic polyneuritis" was observed have been published in other cases it is stated that loss of reflexes persisted as a "residuum." However it seems to be generally agreed that areflexia in diabetics is irreversible. This is also supported by clinical experience, but documented data on this irreversibility are not available

Detailed Information on Areflexia

Sequence

In agreement with WILLIAMSON's observations, more recent investigators have reported that the Achilles-tendon reflexes are lost more frequently and

TABLE 5

The relationship between patellar and Achilles-tendon reflexes in few large series. It is seen that the patellar reflexes are often preserved in patients with loss of Achilles-tendon reflexes, while the reverse is seen in only one case

Authors		No. of patients	Achilles-tendon rflx.				( ) Achilles-tendon rflx.				0 Achilles-tendon rflx.			
			Total	Patellar rflx.		0	Total	Patellar rflx.			Total	Patellar rflx. ( ) 0		
BROCH & KLØVSTAD	(1947)	424	348	347		1					76	32		44
LUNDBÆK	(1953)	89	46	46	0	0	7	7	0	0	36	22	5	9
STEINESS	1957b)	63	25	25	0	0	5	4	1	0	33	15	3	15
FAGERBERG*	(1959)	198	89	89	0	0	10	4	4	2	99	31	20	48

) Patients between 10 and 59 years only

at an earlier stage than the patellar reflexes. This appears clearly from the data compiled in Table 5 in which the combinations of abnormal patellar and Achilles-tendon reflexes in diabetics aged from 10 to 60 years are listed. It is seen that the findings in my own series are in good agreement with those made in other series. GOODMAN & AL. (1953) found similar conditions in their series of 261 patients aged from 10 to 90 years among these patients, loss of patellar reflexes was observed in 76 % and of the Achilles tendon reflexes in 48 % and only two patients showed isolated loss of the patellar reflexes

Systematic studies on the tendon reflexes in the arms in diabetics are not available.

Correlations

Whereas the literature contains many summary surveys of the frequency of abnormal reflexes in large series, only one investigator has performed a detailed study on the occurrence of this isolated neurological phenomenon.

In 270 hospitalised diabetics, KARDING (1956) found sluggish tendon reflexes in 14.4 % and areflexia in 11.5 %. No definite difference as regards age, sex or severity of the disease was observed. The frequency of abnormal reflexes increased with the duration of diabetes, but this increase was not statistically significant.

Even though there are no more detailed reports on the occurrence of abnormal reflexes in patients with diabetes, it is nevertheless possible to throw further light on this problem on the basis of data contained in the literature, since there are a few series with detailed information of each individual patient, which make an analysis possible. These series will be analysed in the same way as my own

Analyses of the Occurrence of Abnormal Achilles Tendon Reflexes

As stated the purpose of these analyses was to ascertain if my series of patients, from a neurological point of view differed from other more or less randomly selected series, and to clarify if the objective neurological abnormalities were correlated with the duration of diabetes.

The reasons for limiting the analyses to comprise only the tendon reflexes were stated in the beginning of this chapter As already pointed out, the Achilles-tendon reflexes are the first to be lost in diabetics, for which reason the analyses are confined to these

In order as far as possible, to avoid neurological abnormalities referable to age—apart from the well-known aged-correlated decrease in vibratory

perception—my series subjected to biothesiometric measurements comprised only patients whose ages ranged from 10 to 60 years. For the same reason the analyses of the other series also comprise only patients in this age range. However for patients over 60 years the data have been listed in the same way as for younger individuals, and these may be considered in an analogous manner

The factors which may primarily be supposed to be of importance in the development of neurological abnormalities are (1) age, (2) sex, (3) duration of diabetes, (4) the severity of the disease (as expressed by the insulin requirements) and (5) the diabetic control.

With a few reservations, there are two series in which the analysis can be fully accomplished viz. those of BONKALO (1950) and FAGERBERG (1959) Furthermore, in the series of BROCH & KLOVSTAD (1947) the data given allow an isolated analysis for a possible correlation between abnormal reflexes and the duration of diabetes, but only for the series as a whole, i.e. also including older patients. Only FAGERBERG's series is so large that it permits a subdivision of the patients who had had diabetes for 15 years or more into two groups, (viz. 15–19 years, and 20 years or more) For comparison with the findings in these two groups of duration may be used the data reported by LUNDBÆK (1953) in his study on long-term diabetics.

The studies of BROCH & KLOVSTAD BONKALO and FAGERBERG do not contain any information as to the diabetic control. Accordingly this factor could not be considered in the analyses i.e. that the diabetic control, if it might be of importance in the development of neurological abnormalities, must be presupposed not to be correlated with any of the other factors.

METHOD OF ANALYSIS

A multidimensional statistical analysis comprising all variables (age, sex, duration and severity of diabetes) would require very large series. The following analyses are therefore limited to an evaluation of the conditions in the various subdivisions of the series.

(1) The composition of the series is illustrated by a division according to age and duration of diabetes, both for the entire series and for the two sexes. For the entire series the results are shown both in tabular and graphic forms (Fig 5 page 58)

(2) The occurrence of abnormal reflexes.

(a) The frequency of abnormal reflexes in each age group and in each group of duration of diabetes is stated in the tables. For the entire series (i.e. men plus women) the result is also represented graphically (Fig 5)

(b) The frequency of abnormal reflexes as related to the insulin requirements is analysed by a division of the series in groups according to the size of the

insulin dose and by subdivisions of these groups according to age and duration of diabetes

In the sections on the analyses and the subsequent discussion the following abbreviations are used —

DD duration of diabetes, in years,
AR, abnormal (Achilles-tendon) reflexes
ID insulin dose.

OWN SERIES

The series comprised 63 unselected patients (38 men and 25 women) whose ages ranged from 11 to 60 years. DD ranged from that of newly discovered disease to 32 years. The series has previously been described in detail (STEINESS 1957b)

The reflexes were studied in the conventional manner if necessary with reinforcement, e.g Jendrassik's manoeuvre If it proved impossible to elicit the reflexes while the patient was lying on a bed the patellar reflexes were studied with the patient in the sitting position while the legs were hanging loosely down, and the Achilles-tendon reflexes with the patient kneeling on a chair If necessary the examination was repeated.

By AR are understood that the procedure used failed to elicit unquestionable Achilles-tendon reflexes. AR were noted in 33 (52%) of 63 patients.

1 *Composition of Series*
See Table 6a.

A. *Age* —There was a relatively large number of elderly patients but the average age in the various DD groups showed no systematic variation (Fig 5)

B. *Duration of diabetes* —The average DD showed no systematic variation in relation to the age groups (Fig 5)

C. *Sex* —Owing to the limited size of the series, it was not divided according to sex in these analyses.

2 *Abnormal Reflexes*
See Table 6a.

A. *Age*—The frequency of AR increased evenly in the first four age groups, but in the oldest patients the frequency was lower than in the preceding two age groups (Fig 5) Owing to the small number of patients in each age group, mere chance may play a part, which should be borne in mind. AR were equally frequent in patients over and under 40 years, viz. 19 (56%) out of 34 and in 14 (48%) out of 29 respectively

TABLE 6.

Own series (STEINESS 1957b)

a. The composition of the series as regards age and duration of diabetes (DD); the frequency of abnormal reflexes (AR) in the various groups (shown graphically in Fig. 5 page 58)

b. The occurrence of abnormal reflexes related the insulin dose (ID) The series is divided according to age and duration of diabetes.

TABLE 6a.

DD	<1	1–4	5–9	10–14	≥15	Total		AR		Average DD
						No.	%	No.	%	
Age										
11–20	1	0	4	0	1	6	10	2	33	8.5
21–30	1	2	2	1	3	9	14	4	44	11.6
31–40	2	2	2	2	6	14	22	8	57	12.1
41–50	2	3	2	2	3	12	19	8	67	10.6
51–60	4	6	4	3	5	22	35	11	50	8.6
Total										
No	10	13	14	8	18	63		33	52	
%	16	21	22	13	29					
AR										
No	1	5	5	6	16	33				
%	10	38	36	75	89	52				
Average age	41	46	37	45	41	42				

TABLE 6b.

ID	0 I.U.		1–40 I.U.		>40 I.U.	
	Total	AR	Total	AR	Total	AR
Age						
11–20	0	0	1	0	5	2
21–30	0	0	3	2	6	2
31–40	1	0	4	1	9	7
41–50	3	1	4	3	5	4
51–60	8	4	7	2	7	5
Total	12	5	19	8	32	20
DD						
< 1	6	1	3	0	1	0
1– 4	4	3	4	0	5	2
5– 9	0	0	4	0	10	5
10–14	2	1	2	2	4	3
≥ 15	0	0	6	6	12	10
Total	12	5	19	8	32	20

B. *Duration of diabetes*—The frequency of AR increased with the DD especially for DD of more than 10 years (Fig 5) Great importance cannot be attached to the difference in the frequencies of AR in the various groups of DD because the number are relatively small, but of the 37 patients with a DD of up to 10 years, 11 (30 %) had AR as against 22 (85 %) of the 26 patients with longer DD this difference is statistically significant (χ^2 test with Yates's correction $P < 0.001$)

3 *Insulin Dose*

On the basis of the ID the disease was divided into three grades of severity (1) mild, no insulin (2) moderate," from 1 to 40 I U of insulin and (3) severe " more than 40 I U of insulin daily The results are shown in Table 6b.

A. *Age*—The common observation, viz. that mild diabetes occur in elderly patients, was confirmed by the analysis. The figures do not suggest any correlation between AR and ID

B. *Duration of diabetes*—This series revealed a tendency to short DD in the mild cases and long DD in the severe cases. The cases of moderate severity showed an even distribution. There was no tendency to a correlation between ID and AR, whereas the previously demonstrated correlation between AR and DD was also observed here.

Thus, the analysis did not reveal any evidence in support of a correlation between AR and ID but the small number of patients did not allow definite conclusions.

BONKALO'S SERIES

BONKALO's series comprised 150 hospitalised patients with no other disease than diabetes. The fact that they were hospitalised did not indicate that their condition was particularly severe since, as a rule, patients who were under the care of the clinic were admitted from time to time for readjusting their treatment. All the patients were receiving good care, and their diabetes was well managed, most of them having been under the supervision of the clinic from the onset of their disease. One of the patients, No 128, was under 10 years of age and was therefore excluded in the present analysis. Of the remaining patients, 108 (73 %) were under 60 years and 41 (27 %) were 60 years or older

The most conspicuous neurological finding was abolished or markedly impaired reflexes on the legs. The Achilles-tendon reflexes were absent in 48 and markedly impaired in 19 i.e. abnormal in a total of 67 patients. The patellar reflexes were absent in fewer viz. 37 but were markedly impaired

TABLE 7

BOVEALO's series (1950).

a. Patients aged from 10 to 59 years. Composition of the series as regards age and duration of diabetes (DD); the frequency of abnormal reflexes (AR) in the various groups (shown graphically in Fig. 5 page 58)

b. The occurrence of abnormal reflexes related to the insulin dose (ID). The series is divided according to age and duration of diabetes.

c. Same as ( ) for patients aged 60 years or over

TABLE 7a. Both sexes

DD	<1	1–4	5–9	10–14	≥15	Total No.	Total %	AR No.	AR %	Average DD
Age										
10–19	2	1	4	0	1	8	7	2	25	5.6
20–29	2	5	4	3	5	19	18	9	47	8.5
30–39	2	6	4	7	5	24	22	11	46	9 6
40–49	4	7	4	6	4	25	23	11	44	7.3
50–59	6	10	2	5	9	32	30	16	50	8.3
Total										
No.	16	29	18	21	24	108		43	49	8.2
%	15	27	17	19	22					
AR										
No	4	9	6	11	19	49				
%	23	31	33	52	79	45				
Average age	42	41	52	41	41	40				

Men

DD	<1	1–4	5–9	10–14	≥15	Total No	Total %	AR No.	AR %	Average DD
Age										
10–19	1	0	1	0	0	2	4	0	0	4.8
20–29	1	4	2	0	1	8	16	4	50	4.9
30–39	0	3	2	2	0	7	14	4	57	6.0
40–49	1	2	4	4	4	15	31	8	53	10.1
50–59	5	6	0	3	5	17	35	7	41	8.9
Total										
No	6	15	9	9	10	49		23	47	8 0
	12	31	18	18	20					
AR										
No	2	5	3	5	8	23				
	33	33	33	56	80	47				
Average age	43	41	54	43	49	42				

Women

DD	< 1	1–4	5–9	10–14	≥ 15	Total No.	Total %	AR No.	AR %	Average DD
Age										
10–19	1	1	3	0	1	6	10	2	33	6.1
20–29	1	1	2	3	4	11	19	5	45	11.1
30–39	2	3	2	5	5	17	29	7	41	11.1
40–49	3	5	0	2	0	10	17	3	30	5.1
50–59	3	4	2	2	4	15	25	9	60	8.2
Total										
No	10	14	9	12	14	59		26	44	8.3
%	17	24	15	20	25					
AR										
No	2	4	3	6	11	26				
%	20	29	33	50	79	44				
Average age	41	42	31	38	36	38				

Table 7b.

ID	0 IU		1–40 IU		> 40 IU	
	Total	AR	Total	+ AR	Total	AR
Age						
10–19	1	0	3	0	4	2
20–29	1	1	4	1	14	7
30–39	1	0	3	1	20	10
40–49	1	0	10	3	14	8
50–59	11	6	8	4	13	6
Total	15	7	28	9	65	33
60–69	4	3	23	14	3	3
70–79	1	0	4	3	2	0
> 80	1	1	1	1	0	0
Total	6	4	28	18	7	3
10–59 years DD						
< 1	4	2	9	1	5	1
1–4	7	3	9	2	15	4
5–9	1	0	3	1	14	5
10–14	2	1	4	3	15	7
≥ 15	1	1	3	2	20	16
Total	15	7	28	9	63	33
> 60 years DD						
< 1	0		1	0	0	–
1–4	1	0	8	4	1	1
5–9	2	1	7	4	0	–
10–14	1	1	7	6	2	0
≥ 15	2	2	5	4	4	2
Total	6	4	28	18	7	3

Table 7

Boveralo series (1950)

a. Patients aged from 10 to 59 years. Composition of the series as regards age and duration of diabetes (DD); the frequency of abnormal reflexes (AR) in the various groups (shown graphically in Fig. 5 page 58).

b. The occurrence of abnormal reflexes related to the insulin dose (ID). The series is divided according to age and duration of diabetes.

c. Same as ( ) for patients aged 60 years or over

Table 7a. Both sexes

DD	1	1–4	5–9	10–14	15	Total		AR		Average DD
						No.	%	No.	%	
Age										
10–19	2	1	4	0	1	8	7	2	25	5 6
20–29		5	4	3	5	19	18	9	47	8.5
30–39	2	6	4	7	5	24	22	11	46	9.6
40–49	4		4	6	4	25	23	11	44	7.3
50–59	6	10	2	5	9	32	30	16	50	8.3
Total										
No.	1[illegible]	29	18	21	24	108		45	49	8.2
	15	27	17	19	22					
AR										
No	4	9	6	11	19	49				
	25	31	33	5[illegible]	[illegible]9	45				
Average age	42	41	32	41	41	40				

Men

DD	1	1–4	5–9	10–14	≥ 15	Total		AR		Average DD
						No.		No.		
Age										
10–19	1	0	1	0	0	2	4	0	0	4.8
20–29	1	4	2	0	1	8	16	4	50	4.9
30–39	0	3	2	2	0	7	14	4	57	6.0
40–49	1	2	4	4	4	15	31	8	53	10.1
50–59	3	6	0	3	5	17	33	7	41	8 9
Total										
No	6	15	9	9	10	49		23	47	8.0
	1[illegible]	31	18	18	20					
AR										
No	2	5	3	5	8	23				
	33	33	33	[illegible]6	80	47				
Average age	43	41	34	45	49	42				

in 30 i.e. abnormal in 67 patients. The tendon reflexes in the arms were markedly impaired or abolished in 29 patients, but details as to the individual patients were not given.

In the tabular survey in BONKALO's paper it is only stated if the patient had abnormal reflexes in the legs. As a total of 74 (50) exhibited AR, seven patients must have had normal Achilles-tendon reflexes, but abnormal patellar reflexes, or vice versa. In this, the findings differ from those of the previously mentioned series (Table 5 page 42)

Of the 108 patients under 60 years, 49 (45) were men and 59 (55) women. Of these, 49 (or 45) had AR. The frequency of AR was of the same order in men and women, viz. 23 (47) and 26 (44)

1 *Composition of Series*

See Table 7a.

A. *Age* —In the entire series (of patients under 60 years) the number of patients increased gradually with advancing age the youngest age group was relatively sparsely represented. In the middle group of DD the average age was about 10 years lower than in the other groups, in which the average age was nearly uniform. There was thus no systematic variation (Fig 5)

The average age of the female group was four years lower than that of the male group. Among the men, there was a relatively large number of elderly patients, whereas the age distribution of the women was more uniform. The difference in age between men and women was most pronounced in the two groups with the longest duration of diabetes.

B. *Duration of diabetes* —In the entire series, the average DD was a little shorter in the sparsely represented youngest age group but in the other age groups it was fairly uniform and without systematic variation (Fig 5)

In the groups for the two sexes the average DD was uniform, although rather heterogeneous in the individual age groups. For the men it was longest in the three oldest age groups while it was uncorrelated with age among the women.

2 *Abnormal Reflexes*

See Table 7a.

A. *Age* —In the entire series, the frequency of AR was uniform in the various age groups, apart from the youngest in which the frequency was about one half of that observed in the remaining groups. In view of the fact that the number of patients in this age group was very small and the average DD shorter than in the other groups, no independent importance can be attached to this deviation.

Among the women, the frequency of AR was highest in the age group of 50–59 years. Even though there was a relatively large number of patients in this age group, the figures are small, and as there was not otherwise any tendency to correlation with age no conclusions can be drawn on this basis.

B. *Duration of diabetes.*—In the entire series, there was no definite difference in the frequency of AR in the three groups with the shortest DD However for a DD of more than 10 years, the frequency of AR showed a distinct increase (Fig 5) Of the 63 patients with a DD of up to 10 years, 19 (30 %) had AR as against 30 (67) of the 45 patients with a DD of more than 10 years. This difference is statistically significant (χ^2 test $P < 0.001$)

In the groups for the two sexes the same relation between AR and DD was revealed.

3 *Insulin Dose*

This was given in international units. On this basis the patients were divided into three groups as described under my own series. The results appear from Table 7b.

A. *Age* —Only the oldest age group contained several patients who did not receive insulin. The numbers of patients in the individual age groups in the group of moderate severity were so small that no weight could be attached to them. The overall results do not suggest any correlation between ID and AR.

A division into two groups according to sex showed the same conditions as the entire series.

B. *Duration of diabetes* —The series revealed a correlation between mild cases and short DD and between severe cases and long DD The number of cases of moderate severity also decreased with increasing DD There was no tendency to correlation between ID and AR, but the previously demonstrated correlation between AR and DD was again observed

The *older patients* (over 60 years) showed a higher frequency of AR than the younger but the DD was also slightly longer They revealed the same correlation between AR and DD as the younger patients, but the figures are small (Table 7c) There was no correlation between ID and AR (Table 7b)

FAGERBERG'S SERIES

FAGERBERG's series comprised a total of 356 diabetics, who were admitted to hospital during the period 1952–1958. In an attempt to make the series as "pure as possible some patients were excluded in the present analysis because the information was incomplete, and a few because of FAGERBERG's

own comments. The patients excluded were Nos. 41 89 110, 118, 136 139 151 153 163 173 174 179 186 216 265 290 323 and 336. Of the remaining patients, 198 (59 %) were under 60 years and 140 (31 %) over 60 years.

By AR are here understood absent or questionable reflexes (indicated by ?)

Of the 198 patients under 60 years, 98 were men and 100 women 54 (55 %) of the men and 55 (55 %) of the women had AR.

As distinct from the preceding series, FAGERBERG's was so large that it was possible to divide the patients with a DD of more than 15 years into two groups viz. DD 15–19 years, and DD 20 years or more.

1 *Composition of Series*

See Table 8a.

A. *Age*—The youngest age group was so sparsely represented that it was not possible to draw isolated conclusions on the basis of these figures. The other age groups were fairly uniformly represented, the oldest being a little larger than the others. The average age in the various DD groups did not show any systematic variation (Fig 5)

The groups of the two sexes presented analogous conditions.

B *Duration of diabetes.*—In the entire series, the average DD was shortest in the youngest age group There was no systematic variation in the average DD in the various age groups (Fig 5)

The groups of the two sexes presented analogous conditions.

2. *Abnormal Reflexes*

See Table 8a.

A. *Age*—In the entire series, the frequency of AR was uniform in the various age groups when the youngest age group for the previously stated reasons was left out of account.

The group of men presented analogous conditions.

In the group of women, the relatively high frequency of AR within the well represented oldest age group was a conspicuous feature (see below) However no systematic variation was revealed.

B. *Duration of diabetes*—In the entire series, the frequency of AR showed distinct increases after DD of 1 and 9 years, respectively The frequency was highest and of the same order of magnitude in the two groups with the longest durations. In view of the conditions mentioned below under the group of women, it is doubtful if the pronounced increase in frequency from the first to the second group of DD is actually so large as found here. Of the 89 patients

TABLE 8.

FAGERBERG series (1959)

a. Patients aged 10 to 59 years. The composition of the series as regards age and duration of diabetes (DD) the frequency of abnormal reflexes (AR) in the various groups (shown graphically in Fig 5, page 58).

b. Same as ( ) for patients aged 60 years or over

c. The occurrence of abnormal reflexes in patients aged 60 years or over related to insulin treatment (no insulin insulin or oral hypoglycaemic agent) The series is divided both according to age and the duration of diabetes.

Of the 198 patients who were under 60 years, only seven received only dietary treatment. The conditions in these patients are discussed in the text (page 57)

TABLE 8a.
Both series

DD	<1	1–4	5–9	10–14	15–19	≥20	Total		AR		Average DD
							No.	%	No.	%	
Age											
10–19	0	3	4	2	0	0	9	4	4	44	6.4
20–29	5	6	6	8	15	6	46	23	27	59	11 7
30–39	8	3	5	6	7	17	46	23	22	48	14 6
40–49	5	4	8	7	5	9	38	19	20	53	12.2
50–59	8	11	13	10	6	11	59	30	36	61	10.2
Total											
No.	26	27	35	33	33	43	198		109	55	11.8
%	13	14	18	17	17	22					
AR											
No.	3	11	14	21	26	34	109				
	12	41	39	64	79	79	55				
Average age	40	41	41	40	35	41	39				

Men

DD	1	1–4	5–9	10–1	5–19	≥20	Total		AR		Average DD
							No.		No.		
Age											
10–19	0	2	2	2	0	0	6	6	3	50	6.7
20–29	3	3	4	2	7	3	22	22	13	59	11 4
30–39	5	2	3	2	5	8	25	[illegible]	14	56	14.2
40–49	4	2	4	2	4	3	19	19	11	58	10.9
50–59	4	1	9	5	1	6	26	27	13	50	10.8
Total											
No.	16	10	22	13	17	20	98		54	55	11.6
%	16	10	22	13	17	20					
AR											
No.	2	2	11	10	14	15	54				
%	13	20	50	77	82	75	55				
Average age	40	32	42	39	34	41	39				

Women

DD	1	1–4	5–9	10–14	15–19	≥ 20	Total		AR		Average DD
							No.	%	No.	%	
Age											
10–19	0	1	2	0	0	0	3	3	1	33	6.0
20–29	2	3	2	6	8	3	24	24	14	58	12.0
30–39	3	1	2	4	2	9	21	21	8	38	13.2
40–49	1	2	4	5	1	6	19	19	9	47	13.4
50–59	4	10	4	5	5	5	33	33	23	70	9.7
Total											
No.	10	17	14	20	16	23	100		55	55	12.0
%	10	17	14	20	16	23					
AR											
No.	1	9	3	11	12	19	55				
	10	53	21	55	75	83	55				
Average age	41	45	39	40	36	41	40				

TABLE 8b. Both sexes

DD	1	1–4	5–9	10–14	15–19	≥ 20	Total		AR		Average DD
							No.	%	No.	%	
Age											
60–69	7	22	12	23	5	5	74	53	51	69	8.3
70–79	8	10	9	12	7	8	55	39	43	78	10.6
≥ 80	2	4	1	2	1	1	11	7	6	55	7.8
Total											
No.	17	36	22	37	13	15	140		100	71	9.2
	12	26	16	26	9	11					
AR											
No.	7	18	18	33	11	13	100				
%	41	50	82	89	85	87	71				
Average age	70	69	69	69	71	73	70				

Men

DD	<1	1–4	5–9	10–14	15–19	≥ 20	Total		AR		Average DD
							No.	%	No.	%	
Age											
60–69	2	8	3	6	0	3	22	56	16	73	8.1
70–79	4	2	2	1	2	4	15	39	12	80	11.7
≥ 80	0	1	0	1	0	0	2	5	2	100	5.5
Total											
No.	6	11	5	8	2	7	39		30	77	9.4
	15	28	13	21	5	18					
AR											
No	4	7	5	7	1	6	30				
	67	64	100	88	50	86	77				
Average age	69	69	69	69	72	70	69				

(Fagerberg series—continued)

Women

DD	<1	1–4	5–9	10–14	15–19	≥20	Total No.	Total %	AR No.	AR %	Average DD
Age											
60–69	5	14	9	17	5	2	52	52	33	67	8.4
70–79	4	8	7	11	5	5	40	40	31	78	10.2
≥ 80	2	3	1	1	1	1	9	9	4	44	8.3
Total											
No.	11	25	17	29	11	8	101		70	69	9 1
%	11	25	17	29	11	8					
AR											
No.	3	11	13	26	10	7	70				
%	27	44	77	90	91	88	69				
Average age	70	69	70	69	71	75	70				

Table 8c.

	0 insulin Total	0 insulin +AR	+ insulin Total	+ insulin +AR
Age				
60–69	22	9	52	42
70–79	12	9	43	34
80–	5	2	6	4
Total	39	20	101	80
DD				
<1	5	3	12	4
1–4	16	7	18	11
5–9	7	5	15	13
10–14	3	1	34	32
15–19	3	2	10	9
20–	3	2	12	11
Total	39	20	101	80

with a DD of less than 10 years, 28 (31 %) had AR, as against 81 (74 %) of the remaining 109 patients this difference is statistically significant (χ test $P < 0.001$)

In the men, the frequency of AR increased evenly through the first five DD groups. Of the 48 patients with a DD of up to 10 years, 15 had AR as against

39 of the 50 patients with a DD of 10 years or more this difference is statistically significant (χ^2 test P < 0.001)

In the female section the analysis revealed a strikingly high frequency of AR in the group with a DD of 1–4 years otherwise the females also showed the highest frequency of AR in patients with long term diabetes. Of 41 patients with a DD of up to 10 years, 13 had AR as against 42 of 59 patients with a DD of 10 years or more this difference is statistically significant (χ^2 test P < 0.001)

Special remarks concerning the women—As already stated the frequency of AR was strikingly high in the age group 50–59 years and in the group with a DD of 1–4 years. A detailed analysis showed that this was due to a very high frequency of AR in the oldest patients with a DD of 1–4 years. Among the 10 patients in this group eight had AR. The conditions of these eight patients were therefore further investigated It appeared that two did not receive insulin four had retinopathy and proteinuria (in one, more than 0.3) one had retinopathy and one had proteinuria. These findings may suggest that some of these patients had diabetes of longer duration than was known (cf LUNDBÆK 1955)

3 *Insulin Dose*

In his tabular survey FAGERBERG only stated whether the patients received insulin (or an oral hypoglycaemic agent) or not.

Of the 198 patients, only seven (all over 40 years) did not receive insulin. These seven were three men with normal reflexes and four women with AR. These figures are too small for any conclusions.

Among the *older patients* (over 60 years) the frequency of AR was distinctly higher than among the younger age groups the average DD was not longer (Table 8b) The correlation between DD and AR observed in younger patients was also seen here but the steep incerase in the frequency occurred earlier after a DD of 5 years.

Of the 140 patients, 39 did not receive insulin or an oral hypoglycaemic agent (Table 8c) Among these 39 patients, 20 (51) had AR. Of the 101 who received insulin, 80 (79 %) had AR. However a subdivision of the series showed that most of the non-insulin treated patients had had diabetes for relatively short periods, as distinct from those who received insulin. The individual subgroups also revealed a correlation between the AR and DD whereas there was no tendency to correlation between AR and insulin treatment.

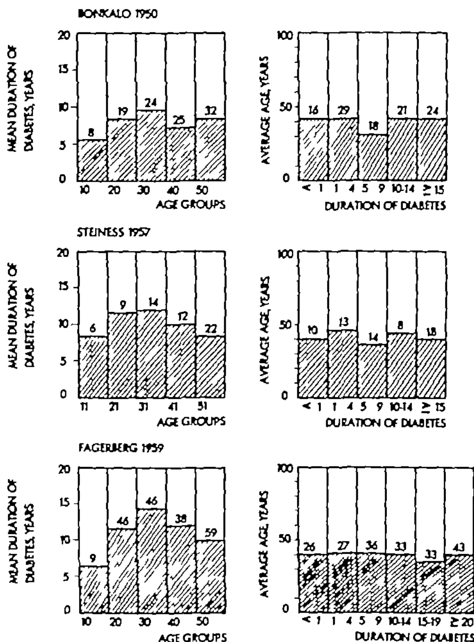


FIG. 5

FIG. 5—The composition of BONKALO's, STEINESS' and FAGERBERG's series. The figures above the columns of the first six diagrams to the left indicate

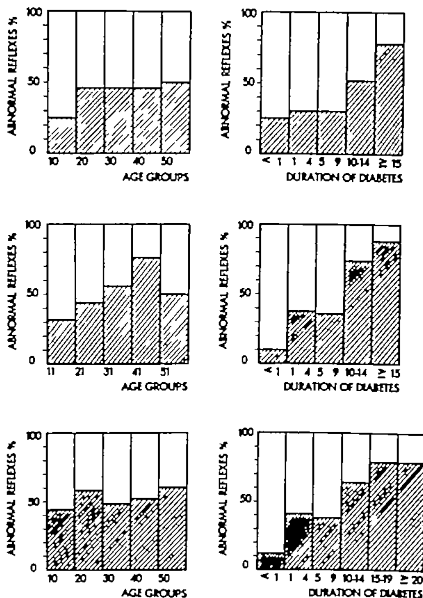


FIG. 5 b

nd the occurrence of abnormal reflexes in the legs in these series.
he numbers of patients. For further details, see the tables.

BROCH & KLOVSTAD'S SERIES

BROCH & KLOVSTAD's series comprised 426 patients who were admitted to hospital or treated as out patients in 1938–1946

Detailed information of each individual patient is only given for the group with diabetic polyneuritis. It was therefore not possible to subdivide the series to the same extent as was done in the preceding series, but the frequency of loss of Achilles-tendon reflexes can be analysed in relation to the groups for DD used by the authors.

In the present analysis, two patients with "diabetic polyneuritis" were excluded because of the comments given in the paper (No 20 Acromegaly Myxoedema. Diabetes mellitus sanata No. 30 Ischias. Osteochondrosis intervertebralis)

Of the remaining 424 patients, 164 (39 %) were under and 260 (61) over 50 years of age. AR were observed in 76 patients (18%) i.e. this frequency was somewhat lower than in the preceding series.

TABLE 9

BROCH & KLOVSTAD series (1947)
The occurrence of abnormal reflexes (AR) related to the duration of diabetes (DD)

DD	< 1	1–5	6–10	> 10	Total
No. of patients	60	125	130	109	424
AR					
< 50 years	0	2	11	7	20 of 164 (12%)
> 50 years	4	10	17	25	56 of 260 (22)
Total	4	12	28	32	76
%	7	10	22	29	18

From Table 9 it appears that the frequency of AR, in agreement with the observations in the preceding series, increased with the DD In view of the composition of the series, as it appears from the table it was found to be reasonable to divide it into two groups with a DD up to 6 years, and 6 years or more while 10 years was used as the limit in the preceding groups. Of 185 patients with a DD of up to 6 years, 16 showed loss of the Achilles-tendon reflexes as against 60 of 239 patients with a longer DD this difference is statistically significant (χ^2 test $P < 0.001$)

As in the preceding series, the frequency of AR was highest in the older patients. However as the composition as regards DD in the two age groups cannot be ascertained, it is impossible to decide whether this finding is a reality or if there may be a false correlation because of a longer DD in the older patients.

However with the exception of the lower frequency of AR, this series does not differ from the preceding ones.

LUNDBÆK'S SERIES

LUNDBÆK's series consisted of 165 patients in whom the DD ranged from 15 to 25 years. It comprised all diabetics who had their residence within a certain area (the Municipality of Aarhus) during a certain period. Information as to the absence or presence of reflexes is not given in five cases. Of the remaining 160 patients, 89 (56) were under 60 years while 71 (44%) were over 60 years.

By AR are here understood absent or questionable reflexes, the latter finding being marked by parentheses in the table

1 *Composition of Series*

See Table 10a

A. *Age*.—Apart from the youngest age group, which comprised only one patient, the others were of the same order of magnitude with a slightly increasing representation with increasing age The average age was slightly higher for the patients with the longest DD but yet of the same order of magnitude

The composition of the groups for the two sexes was uniform.

B *Duration of diabetes*.—The average DD was practically the same in the various age groups.

The groups for the two sexes were almost identical.

2. *Abnormal Reflexes*

See Table 10a.

A. *Age*.—In the entire series, no correlation between age and AR was revealed.

The frequency of AR was alike in men and women. The oldest male patients showed a somewhat higher frequency of AR, but as the figures are relatively small, no importance can be attached to this observation.

B *Duration of diabetes*.—The frequency of AR was alike in the two groups with DD 15–19 years and DD 20–25 years.

3 *Insulin Dose*

The patients were divided into three groups according to the size of the ID in I U., in the same way as described in the section on my own series. The results appear from Table 10b

BROCH & KLOVSTAD'S SERIES

Broch & Klovstad's series comprised 426 patients who were admitted to hospital or treated as out patients in 1938–1946.

Detailed information of each individual patient is only given for the group with "diabetic polyneuritis." It was therefore not possible to subdivide the series to the same extent as was done in the preceding series, but the frequency of loss of Achilles-tendon reflexes can be analysed in relation to the groups for DD used by the authors.

In the present analysis, two patients with "diabetic polyneuritis" were excluded because of the comments given in the paper (No. 20 "Acromegaly. Myxoedema. Diabetes mellitus sanata." No. 30 "Ischias. Osteochondrosis intervertebralis").

Of the remaining 424 patients, 164 (39%) were under and 260 (61%) over 50 years of age. AR were observed in 76 patients (18%), i.e. this frequency was somewhat lower than in the preceding series.

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Total	4	12	28	32	76
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From Table 9 it appears that the frequency of AR, in agreement with the observations in the preceding series, increased with the DD. In view of the composition of the series, as it appears from the table, it was found to be reasonable to divide it into two groups with a DD up to 6 years, and 6 years or more, while 10 years was used as the limit in the preceding groups. Of 185 patients with a DD of up to 6 years, 16 showed loss of the Achilles-tendon reflexes as against 60 of 239 patients with a longer DD; this difference is statistically significant (χ^2 test, $P < 0.001$).

As in the preceding series, the frequency of AR was highest in the older patients. However, as the composition as regards DD in the two age groups cannot be ascertained, it is impossible to decide whether this finding is a reality or if there may be a false correlation because of a longer DD in the older patients.

Women

DD	15–19	20–25	Total		AR		Average DD
			No.	%	No.		
Age							
10–19	0	0	0		–	–	–
20–29	6	2	8	20	2	25	18.1
30–39	5	4	9	22	6	66	20.2
40–49	6	4	10	24	4	40	19.2
50–59	6	8	14	34	5	36	21.2
Total							
No.	23	18	41		17	41	19.9
	56	44					
AR							
No.	9	8	17				
%	39	44	41				
Average age	41	44	42				

Table 10b.

ID	I.U.		1–40 I.U.		> 40 I.U.	
	Total	AR	Total	+ AR	Total	+ AR
Age						
10–19	0		0		1	1
20–29	0		3	1	15	6
30–39	0		7	6	15	5
40–49	0		8	4	15	6
50–59	4	3	10	5	13	6
Total	4	3	28	16	57	24
60–69	12	5	22	14	11	9
70–79	5	3	15	10	6	5
80-	0		0		0	
Total	17	8	37	24	17	14
10–59 years						
DD						
15–19	3	2	15	9	29	13
20–25	1	1	13	7	28	11
Total	4	3	28	16	57	24
≥ 60 years						
DD						
15–19	11	5	22	15	5	5
20–25	6	3	15	9	12	9
Total	17	8	37	24	17	14

(Lenomax series—continued)

Table 10c. Both sexes

DD	15–19	20–25	Total No.	Total	AR No.	AR	Av
Age							
60–69	28	17	45	63	28	62	
70–79	10	16	26	37	18	69	
80–	0	0	0		–		
Total							
No.	38	33	71		46	65	
%	54	46					
AR							
No.	25	21	46				
%	66	64	65				
Average age	67	68	68				

Men

DD	15–19	20–25	Total No.	Total	AR No.	AR	Av
Age							
60–69	12	10	22	66	10	45	
70–79	3	8	11	33	9	82	
80–	0	0	0				
Total	15	18	33		19	58	
	45	55					
AR							
No.	7	12	19				
%	47	66	58				
Average age	66	68	67				

Women

DD	15–19	20–25	Total No.	Total	AR No.	AR	Av
Age							
60–69	16	7	23	61	18	78	
70–79	7	6	13	39	9	69	
80–	0	0	0				
No.	23	13	38		27	71	
	61	39					
AR							
No.	18	9	27				
%	78	60	71				
Average age	68	69	68				

A. *Age*—Division into age groups did not reveal any *correlation between* ID and AR

B. *Duration of diabetes*—Division of the series according to DD did not either show any correlation between ID and AR

In the *older patients* (over 60 years) the frequency of AR was distinctly higher than among the younger patients. The average DD was alike in the older and younger patients. The frequency of AR showed a distinct increase with the ID Of 17 patients who had not received insulin eight (47 %) had AR as against 38 (70 %) of the 54 insulin treated patients however this difference is not statistically significant (χ^2 test $P > 0.5$)

DISCUSSION OF CORRELATION ANALYSES

The analyses revealed very good agreement between my own series and those of BONKALO and FAGERBERG both as regards the composition of the series and the distribution of AR.

In all three series, the frequency of AR showed a statistically significant correlation with the DD and no signs were revealed which would suggest the existence of a false correlation with the duration Thus correlation between AR and DD is further supported by the findings in the series of BROCH & KLOVSTAD In view of these findings, it must be justified to regard my own series as representative from a neurological point of view and to consider the correlation between AR and DD as a reality

The size of my series did not allow a subdivision according to sex with the method of analysis used. However as the series described by BONKALO FAGERBERG and LUNDBÆK did not reveal any difference between the two sexes, it must be concluded that diabetics do not exhibit any sex differences as regards AR. In this connexion it must be appropriate to recall that neither did diabetics reveal any sex differences as regards impairment in vibratory perception.

Both my own series and that of FAGERBERG give the immediate impression that the frequency of AR shows a steep increase after a DD of 1 year (Fig 5 page 58) As the frequency of AR is of the same order of magnitude in the groups DD 1–4 years and DD 5–9 years in all three series (STEINESS, FAGERBERG and BONKALO) the relatively high frequency of AR in recent cases of diabetes in BONKALO's series might be explained as being due to mere chance. However it must be pointed out that the number of patients in my own series is not so large that it is permissible to draw independent conclusions for each individual group of duration but, obviously this does not exclude that it may be possible to see if certain tendencies are present. In addition, as previously mentioned there may be reasons to believe that female patients with AR in the group DD 1–4 years in FAGERBERG's series are overrepre

sented. Thus there is no definite evidence in support of the assumption that the frequency of AR should increase steeply after a DD of 1 year and then remain unchanged up to a DD of 10 years. The series considered do not exclude that a gradual increase in the frequency may occur for which reason this question must remain unanswered.

It appears from Figure 5 that the frequency of AR according to FAGERBERG's findings is alike at a DD of 15–19 years and in diabetes of longer duration. Even though the frequency of AR in LUNDBÆK's series of long term diabetics was lower than that reported by FAGERBERG among similar patients, LUNDBÆK's series did not reveal any difference in the frequency of AR in the two groups DD 15–19 and DD 20–25 years. This may imply either that the frequency of AR does not increase after a DD of 15 years or that a selection occurs due to a higher mortality among the patients with AR, just as was emphasized by LUNDBÆK (1959) as regards retinopathy. From a biological point of view the latter possibility seems to be more reasonable.

The entire series did not reveal any evidence in favour of a correlation between AR and age within the range from 10 to 60 years. However in the youngest age group the numbers of patients were so small that the result must be taken with reserve. Both in BONKALO's and FAGERBERG's series, the female groups showed a strikingly high frequency of AR in the age group 50–59 years. As just stated, in BONKALO's series this may be due to mere chance as the number was small, viz. 15. However in FAGERBERG's series, the number of female patients in the age group 50–59 viz. 33 was larger than any of the other age groups, while the average DD was shorter than in the other female age groups, apart from the youngest group. However closer investigation on this point suggested that some of these women might have had diabetes for a longer period than was known. If thus be the case, it may explain the high frequency of AR in this age group, and if so there is no reason to believe that these women should have an increased frequency of AR. This view is supported by the fact that LUNDBÆK's series did not reveal any signs of such an increased frequency.

On the other hand, in patients over 60 years of age, the frequency of AR was distinctly higher than among younger patients in all series (BONKALO, FAGERBERG, LUNDBÆK) and only in BONKALO's series was the average DD longer than in the younger patients. This is in agreement with the general conception that diabetic neuritis is most common in elderly patients (e.g. JORDAN 1936, BROCH & KLOVSTAD 1947, AARSETH 1953, HIRSON & AL. 1953).

It cannot be decided here why AR are more frequent in older than in younger diabetics. There are three possible explanations. (1) The adult type of diabetes which is most frequently encountered in a group of elderly patients may be more apt to result in nerve lesions than the juvenile type of diabetes.

It is generally agreed that "diabetic neuropathy" in children is very rare, if it does occur at all. The cause of this is unknown. However it appears that patients in whom diabetes has developed early in life will later suffer from neurogenic affection just as all other diabetics. Thus, among 478 juvenile diabetics who had had the disease for more than 30 years, WHITE (1960) found peripheral neuropathy in 48. The distinct correlation between DD and AR revealed in the preceding analyses offers a good explanation of the fact that children do not present objective signs of neurogenic affection and is thus fully compatible with WHITE's observations. (2) It is a common view that the reflexes tend to disappear with advancing age. Thus, MÖBIUS (1883) found abolished or impaired patellar reflexes in one third of 56 persons over 80 years. However, GOODMAN & AL. (1953) observed loss of one or more of the tendon reflexes in the legs in only three of 56 non-diabetics aged from 55 to 70 years, and in four of 27 persons over 70 years. Among 100 elderly chronic in patients whose ages averaged 73 years, HIRSON & AL. (1953) found that the Achilles-tendon reflexes were absent in 42 and impaired in 19. (3) Among elderly patients, there will always be some with very mild diabetes. It is well known that in these patients it will often be difficult or impossible to ascertain the time of onset of the metabolic disorder. In such cases, a correlation with the duration will become blurred.

Whatever the cause may be, the higher frequency of AR in elderly diabetics with a short DD will, in series comprising both younger and older diabetics, result in a reduction in the percentual difference in the frequency of AR in the various groups of duration. This will especially occur in series with a large proportion of elderly patients, as is usually seen in the published unselected series. However, in spite of this, it was possible to demonstrate a statistically significant correlation between AR and DD in BROCH & KLOVSTAD's series, but it is possible that this inclusion of old patients explains why KAEDING, although he observed that the frequency of AR increased with the DD, failed to demonstrate a statistically significant correlation.

The present analyses did not show any correlation between AR and ID. The frequent occurrence of AR in elderly patients, among whom mild cases of diabetes are strongly represented, is also against the assumption of such a correlation. This findings is at variance with the results of BROCH & KLOVSTAD and MATTHEWS, but in their surveys no regard was paid to the possible influence of the DD.

In conclusion it may be said that the investigations described have shown that the frequency of AR in the legs in diabetics has not decreased since the introduction of insulin treatment.

The analyses performed have shown that the frequency of AR is correlated

with the DD No correlation was revealed between AR and age in the range from 10 to 60 years, but AR were more frequent in elderly diabetics. No sex difference could be demonstrated, and there was no correlation with the severity of the disease as expressed by the size of the ID

These analyses were performed on the presupposition that good or poor control of the diabetes, if this might be of importance, was not correlated with any of the other factors in the series concerned.

As my own series showed good agreement with those reported by other investigators, it must be regarded as being representative, and it should thus also be justified to claim that the results of my biothesiometric studies are of general application.

CHAPTER 4

Conclusive Considerations on the Pathogenesis of Neurogenic Affection in Diabetics

The observation that vibratory perception on the legs decreases with increasing duration of diabetes (STEINESS 1957b) was further substantiated in Chapter 3 and it was shown that the occurrence of abnormal reflexes is correlated with the duration of diabetes. The close relationship between a high vibratory perception threshold and abnormal reflexes in diabetics is in favour of the assumption that it is the same pathogenic factor which is the cause of the impairment of both these functions.

In addition to the nerve lesions which occurred after diabetes of fairly long duration, the biothesiometric measurements also suggested that a nerve lesion exists which is unrelated to the duration of the metabolic abnormality. The vibratory perception threshold on the index fingers was thus significantly increased, although no correlations could be demonstrated. Accordingly it seems to be a question of a "concomitant" threshold increase in the diabetic disorder.

At the present time opinions on the pathogenesis of "diabetic neuropathy" are divergent. Some investigators consider this lesion to be of vascular origin, while others believe that the neurological abnormalities are due to metabolic factors. The results of biothesiometric measurements suggest that both a vascular and a metabolic factor may be involved. Evidence is available in favour of the assumption that the nerve lesion which is correlated with the duration of diabetes is due to vascular factors, and, at the same time, it is most reasonable to assume that the "concomitant" threshold increase is caused by metabolic abnormalities, even though it was not possible, with the technique used, to demonstrate any correlation between the vibratory perception threshold and the severity of the disease or the immediate diabetic regulation.

The purpose of this chapter is to discuss if the results of other studies are compatible with the assumption of a dual genesis of the nerve affection in patients with diabetes, as was suggested by the results of the biothesiometric measurements.

It was previously a widespread conception that the neurological abnormalities in diabetics were wholly or partially due to the frequent occurrence

of "arteriosclerosis" in these patients (e.g., WOLTMAN & WILDER 1929 JORDAN 1936 DRY & HINES 1941 KAUVAR 1941 TREUSCH 1945 BROCH & KLOVSTAD 1947)

However it does not any longer seem reasonable to maintain the assumption that "arteriosclerosis"—in the sense of vascular disease with demonstrable peripheral insufficiency—of the lower extremities per se should be the cause of neurological abnormalities in patients with diabetes. The study published by WOLTMAN & WILDER in 1929 has often been cited in support of this hypothesis but in 1940 WILDER revised this view because of the rarity of subjective symptoms of "neuritis" in arteriosclerotic non-diabetics as distinct from their high incidence in diabetics. HIRSON & AL. (1953) studied 150 patients with diabetes they did not find any evidence in support of the assumption that "arteriosclerosis" should be of pathogenic importance they observed both neurological abnormalities in patients without "arteriosclerosis" and also patients with "arteriosclerosis" without neurological symptoms.

If loss of Achilles-tendon reflexes be accepted as a sign of neurogenic affection in diabetics it is evident that the hypothesis of "arteriosclerosis" becomes untenable, since this areflexia is observed in many diabetics without demonstrable signs of arterial insufficiency Thus, LUNDBÆK (1953) observed loss of the Achilles-tendon reflexes in many patients who had had diabetes for from 15 to 25 years, and in whom pulsation of the dorsal artery of the foot was present (cf. l c., Table 10 page 138) and as distinct from occlusive phenomena the abnormal reflexes were not correlated with age. In five of eight patients with "the neuropathic syndrome," i.e. with unquestionable signs of neurogenic affection in the form of diminished tactile sensibility on the feet and lower legs, pulsation of the dorsal artery of the foot was present.

Further evidence against the assumption of the "arteriosclerotic" pathogenesis is provided by the fact that diabetics with trophic lesions of the feet often show unimpaired function of the large vessels, but usually present demonstrable neurological abnormalities—in particular impaired pain sensation (OAKLEY & AL. 1956 J PEDERSEN 1960) In this connexion it is of interest to recall that most patients with diabetic arthropathy who usually exhibit signs of neurogenic affection, reveal normal pulsation of the dorsal artery of the foot to exemplify this, it may be mentioned that BAILEY & ROOT (1947) found normal pulsation in 14 out of 17 of this type of patients, of whom 14 revealed evidence of diabetic neuropathy

While "arteriosclerosis"—in the sense of demonstrable occlusive vascular disease in the legs—thus cannot per se be the cause of neurogenic affection in diabetics, several correlations suggest the existence of a relationship between the specific *diabetic angiopathy* (LUNDBÆK 1953 1954) and objective

neurological abnormalities in diabetes. It is a well-known fact that retinopathy nephropathy and diabetic neuropathy often occur simultaneously (so-called "diabetic triopathy") the frequent combination of retinopathy nephropathy and objective neurological abnormalities in patients under 50 years appears distinctly from the illustrations in FAGERBERG's paper (1959). In this connexion it is therefore of great interest to note that there was no sex difference as regards the vibratory perception on the feet of patients with diabetes as contrasted with non-diabetics (STEINESS 1957b). In diabetics, the sex ratio for vascular abnormalities is also 1 1 (LUNDBÆK 1953) while vascular disease involving the heart and the lower extremities in non-diabetics is more frequent in men than in women within identical age groups. The results of the biothesiometric measurements are thus compatible with the hypothesis that the nerve lesion in diabetics is at least partially due to diabetic angiopathy of the vasa nervorum.

The aforementioned statistical correlations can obviously no more than other statistical correlations prove a causal relationship but can only be taken as supportive evidence. It is therefore of significance that *histological examination* of the peripheral nerves in diabetics has now shown that the pathological prerequisites for a vascular genesis are present. In biopsy specimens of nerves from the legs FAGERBERG (1959) observed a close relationship between objective neurological manifestations and histological changes in the vasa nervorum. The changes consisted in hyalinisation, reduction of the lumina, thickening of the walls and a positive PAS-staining reaction of the vasa nervorum in diabetics. GOLDENBERG & AL. (1959) observed similar changes in amputation specimens from diabetics who did not suffer from hypertension and found agreement between clinical and histological abnormalities in the vasa nervorum. WOLTMAN & WILDER (1929) also revealed marked thickening of the walls of the intraneural vessels in nine of their ten amputation specimens, but by the histological technique used at that time it was not possible to differentiate between specific diabetic changes and other vascular disorders.

Histological studies of the peripheral nerves aiming at disclosing which fibres sustain the greatest injury in diabetes are very sparse. MARTIN (1953b) found a greater reduction in the number of non-myelinated than in the myelinated fibres in ten biopsy specimens. FAGERBERG (1959) performed histological studies with a special view to degeneration of the myelin sheaths and found good agreement between this manifestation and the clinical signs of neuropathy. Further investigations within this field are required.

The mechanism of a vascular affection must consist in nutritive lesions referable to inadequate blood supply. Accordingly the possibility existed that

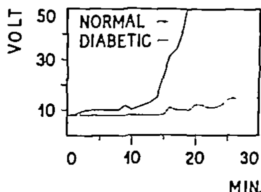


Fig. 6.—The vibratory perception threshold in the great toe during ischaemia in a non-diabetic subject and markedly abnormal curve in diabetic patient (Steiness 1959).

Abscissa Time in minutes from the end of the inflation of the cuff.
Ordinate Vibratory perception threshold, expressed in terms of volts.

It is seen that the vibratory perception decreased only slightly during the half hour in the diabetic patient.

induction of *artificial ischaemia*, for example, by inflation of a pneumatic cuff around the thigh to a level above the systolic blood pressure might reveal an incipient nerve affection, which was still clinically latent. In non-diabetics, inflation of a pneumatic cuff around the thigh results in a depression of the sensibility on the toes after a period of 10 to 15 minutes, and this depression gradually spreads proximally It might therefore be assumed that this sensory disturbance would occur earlier in diabetics than in non-diabetics. By means of a biothesiometer it would be possible to obtain a quantitative measure of the disturbance.

However contrary to expectation, biothesiometric measurements of the vibratory perception threshold during ischaemia revealed that diabetics were often able to perceive the vibrations on the great toes for longer periods than non-diabetics, and in some diabetics the threshold remained almost unchanged during an experimental period of 30 minutes (Figs. 6, 7 and 8) (Steiness 1959) The period during which the vibrations could be felt—the time of perception"—was fairly constant in non-diabetics, and it was not possible to change the time of perception in the diabetic direction by hyper glycaemia induced by an intravenous injection of glucose, by a diet low in carbohydrates or fasting or by administration of cortisone. This suggests that the abnormal conditions in diabetics are due to special factors in this disease (Steiness 1961 a) Continued studies showed that the abnormal conditions in diabetics were reversible independent of the duration of diabetes, but correlated with the immediate diabetic status (Steiness 1961 b)

Even though it cannot be doubted that vibratory perception during ischae mia depends on the immediate diabetic status, it was not always possible to demonstrate a close correlation between the severity of the metabolic abnor mality as assessed by the usual criteria and the vibratory abnormality Even in the presence of an apparently satisfactory diabetic status, several patients revealed an abnormal ischaemia curve. However in such cases, more or less

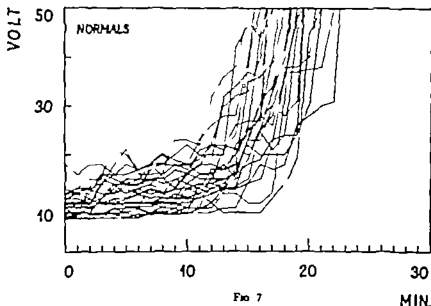


Fig. 7

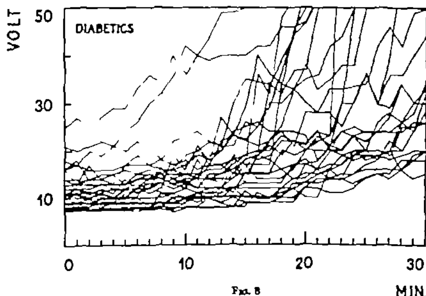


Fig. 8

Figs. 7 and 8.—The vibratory perception threshold in the great toe during ischaemia in 24 normal subjects and 27 diabetics (Steiness 1959).

Abscissa: Time in minutes from the end of the inflation of the cuff.
Ordinate: Vibratory perception threshold, expressed in terms of volts.

In non-diabetics the threshold begins to increase after 10–15 minutes, and none of the subjects could perceive the vibrations in the great toe after 22.5 minutes. In many of the diabetics the threshold increased only slightly during the 30 minutes. Incidentally diabetics reveal all transitions from perfectly normal to severely abnormal curves.

normal curves could be obtained by intense insulin treatment, but the doses administered should be so large that they could not be maintained for a longer period. It must also be mentioned that one of the patients (Case 12 STEINESS 1961b) who at the first test revealed unquestionable subjective symptoms of insulin overdosage, at that time presented a somewhat irregular ischaemia curve with an abnormally long time of perception. After reduction of the insulin dose a normal curve was obtained further studies in this patient showed the usual correlation between the diabetic status and vibratory perception Mention must also be made of the fact that another patient (Case 10) showed normal curves in spite of a very poor diabetic status.

The neurophysiological background against which these ischaemia tests should be assessed may be summarised as follows (1) The prerequisite for perception of vibrations on the great toe is an intact impulse conduction from the toe to the brain. (2) The sensory disturbances during ischaemia is due to an interruption of the nerve conduction at the place where the pneumatic cuff is applied (LEWIS & AL. 1931) (3) The prerequisite for the conduction of an impulse through a nerve is the maintenance of a membrane potential. This requires energy and as reviewed by BRINK (1957) there is every reason to believe that axonal chemistry resembles that of other cells.

When diabetics are able to perceive the vibrations for a longer period than non-diabetics, this must be due to the fact that their nerves are capable of maintaining a membrane potential during ischaemia for a longer period than is normal. The ischaemia tests have thus showed that the hormonal status is of significance in the metabolism of the axons, but they do not reveal whether or not the abnormal conditions are present in the absence of the unphysiological conditions prevailing during arrested blood flow

At the present time, it is hardly possible to offer a chemical explanation of the underlying conditions on which this phenomenon is based but it is reasonable to associate it with insulin. It is therefore interesting to note that it has been possible to demonstrate a direct *effect of insulin* on isolated sciatic nerves from rats as regards uptake of glucose (RAFAELSEN 1958) and oxygen (HELLER & HESSE 1960)

As just mentioned, the abnormal functional conditions which may be observed in the patients during ischaemia do not involve that functional disturbances are also present outside the period of artificial ischaemia. It is therefore of very great interest that recent investigations have shown that the *conduction velocity of motor nerves* is reduced in diabetics (SKILLMAN & AL. 1961) A priori, there is no reason to believe that motor nerves should differ materially from the sensory nerves conducting vibratory impulses both types consist of thick myelinated fibres. As might be expected in view of this,

SKILLMAN & AL. found good agreement between sensory neuropathy and impairment of the conduction velocity of motor nerves However the mode of presenting the findings makes the interpretation of the results difficult from the present point of view On the face of it it might appear that reduced nerve-conduction velocity and duration of diabetes are correlated, since the nerve-conduction velocity is reduced in all types of angiopathy which are known to be correlated with the duration of diabetes. On the other hand it is difficult to reconcile the following findings with a correlation with the duration of diabetes and hence with a vascular genesis (1) Patients with distressing subjective neuropathy but without objective neurological signs revealed the greatest average reduction in the conduction velocity (2) Patients with objective neuropathy may have normal nerve-conduction velocity (3) The nerve-conduction velocity was frequently found to be reduced in the arms, in which clinical neurological abnormalities are rare. MULDER & AL. (1961) also observed slowing of the conduction velocity both in arms and legs in diabetics who did not suffer from neuropathy objective neurological signs were required as a criterion for a diagnosis of neuropathy However patients with neuropathy showed a greater reduction in the nerve-conduction velocity than those without neuropathy

It might be conceived that the reduction in the conduction velocity in patients with diabetes is due to ischaemia referable to diabetic angiopathy of the vasa nervorum or to a specific diabetic metabolic abnormality On the basis of the aforementioned studies it cannot be decided whether the reduced nerve-conduction velocity is correlated with the duration of diabetes or with the immediate diabetic status, or perhaps, with both. However the assumption of a diabetic metabolic component as a causative factor in the genesis of this abnormality is supported by the fact that ELIASSON (1961) found a definite slowing of peripheral nerve conduction in rats soon after the induction of diabetes with alloxan. Further clinical studies are required in order to clarify the pathogenesis. The results of the biothesiometric measurements of vibratory perception during ischaemia suggest that in the search of a solution to this problem it will be most appropriate to perform repeated measurements in the same individuals during variations in the diabetic status, since the criteria in common use for the evaluation of the diabetic status do not seem to give an adequate expression of the "cellular diabetic status. It would therefore be of particular interest if such studies were combined with measurements of the nerve-conduction velocity during ischaemia and simultaneous measurements of the vibratory perception.

It appears difficult to explain the *concomitant" increase in the vibratory perception threshold* on the fingers, for which no correlations with the duration of

diabetes or with the immediate diabetic status could be demonstrated. The latter observation speaks against the assumption of a metabolic cause, although this would be the most reasonable explanation. However it must be emphasised that the metabolic abnormalities seem to be very complex. Thus, the ischaemia tests showed that there may be a pronounced dissociation between the "humoral diabetic status," as expressed by the blood-sugar level, and the "cellular diabetic status," expressed by the ischaemia curve, and that there are wide individual variations in the mode of reaction. If the metabolic abnormalities which cause the "concomitant" increase in the perception threshold are equally complex, this may perhaps, explain why it was not possible to demonstrate any correlation between the threshold increase and the diabetic status.

The ischaemia tests showed that the metabolic status plays a part in the metabolism of the peripheral nerves, and the studies on nerve-conduction velocity suggest that metabolic abnormalities exert an influence on the function of the peripheral nerves. The theoretical prerequisite for the hypothesis of a metabolically conditioned "concomitant" threshold increase is thus present. However this does not exclude the possibility that the impairment of vibratory sensation on the fingers may also progress with the duration of diabetes. On the contrary such a correlation must actually be expected, but as the neurological disturbances in diabetics principally involve the legs it is reasonable to assume that this will also be the case as far as vibratory perception is concerned. That it was not possible to demonstrate an impairment in the vibratory perception on the fingers which was correlated with the duration of diabetes may to some extent be due to the fact that it had been blurred by a "concomitant" threshold increase.

With the exception of sequelae of vascular insults of the central nervous system, all data suggest that the neurological abnormalities observed in the clinical examination of patients with diabetes are referable to lesions of the peripheral nerves. It is true that no specific pathological lesions of the central nervous system directly referable to diabetes have been demonstrated, but it is equally true that systematic investigations with a view to this have never been performed in large series of patients. On the other hand, *electro-encephalographic studies* on large series are available in which it is concordantly shown that dysrhythmia occurs in more than 50 % of the patients with repeated severe insulin reactions (GREENBLATT & AL. 1946 IZZO & AL. 1953 KRUMP 1959) It is still an unsolved problem if dysrhythmia occurs more frequently in other diabetics than in normal subjects, but the changes are at least very slight.

The close relationship between the impairment in vibratory perception on the legs, which is correlated with the duration of diabetes, and loss of the ten-

don reflexes in the legs is in favour of the assumption of a peripheral genesis of this component of the vibratory impairment. In view of the demonstrated hormonal influence on the peripheral nerves it is conceivable that a peripheral genesis of the "concomitant" threshold increase may exist, but it does not exclude the possibility of a central functional disturbance.

Previously it was generally agreed that *insulin* did not exert any direct influence on the carbohydrate metabolism of the central nervous system, but RAFAELSEN (1958, 1961 a, b) has recently demonstrated a significant insulin effect on the uptake of glucose by the isolated spinal cord of rats and by the first superficial pia-covered slice of cerebral sections. Even though it has not so far been possible to demonstrate definite functional disturbances in the central nervous system in patients with diabetes, the results of RAFAELSEN's studies show that this possibility must be included in the considerations concerning the genesis of the "concomitant" increase in the vibratory perception threshold.

Summary

Chapter 1

DIABETIC NEUROPATHY

A brief survey is given of the most frequent neurological abnormalities which are described in the literature on "diabetic (poly)neuritis" or "diabetic neuropathy."

It is generally agreed that the most frequent neurological manifestations in patients with diabetes are localised to the lower extremities but considerable diversity of opinion exists as to the delimitation of the concept of "diabetic neuropathy". The subjective symptoms are often very difficult to evaluate; during recent years there has therefore been a tendency to restrict the term "diabetic neuropathy" so as to comprise only objective neurological manifestations.

The statements as to the frequency of "diabetic neuropathy" vary within wide limits, to some extent due to variations in the criteria for the diagnosis. Opinions are also divergent as to whether "diabetic neuropathy" is correlated with the severity, regulation and duration of the disease and with the age of the patient. These differences of opinion may partially be explained by variations in the criteria for the diagnosis while in other cases the explanation may be that no regard has been paid to the possibility of false correlations caused by the composition of the series. Most studies are only sparsely documented and accordingly the results cannot be subjected to a closer evaluation.

Chapter 2

VIBRATORY PERCEPTION IN DIABETICS

Vibratory perception can be studied by means of a tuning fork or a biothesiometer which is a simple electromagnetic apparatus.

Tuning fork tests are beset with a number of fundamental sources of error. Impairment in vibratory perception may easily be diagnosed in too small a number of young individuals or in too large a number of older individuals, who may have high physiological thresholds. The results of tuning fork tests obtained in diabetics are discussed.

Biothesiometric tests give a direct measure of the threshold of the vibratory sensation, usually arbitrarily expressed in volts. Biothesiometric measurements in diabetics are reported. In my own series (STEINESS 1957b) a statistically significant correlation between a high vibratory perception threshold on the feet and the duration of diabetes was demonstrated. This finding is supported by other studies in which the results were not subjected to statistical analysis. In contrast to this, MIRSKY & AL. (1953) did not find any correlation with the duration of diabetes in a large series of patients in which statistical analysis was employed.

It was shown that the vibratory perception threshold on the index finger is significantly increased in diabetics, but it was not possible to demonstrate any correlations.

The occurrence of other objective neurological manifestations also showed a statistically significant correlation with the duration of diabetes in my series, but not in that described by MIRSKY & AL. Thus, the two series differ from a neurological point of view.

CHAPTER 3

ABNORMAL REFLEXES IN DIABETICS

It is generally agreed that diminished or abolished tendon reflexes, together with impaired vibratory perception, are early objective findings in patients with diabetic neurogenic affection. The Achilles-tendon reflexes are the first deep reflexes to be lost. The frequency of abnormal reflexes has not decreased since the introduction of insulin.

In my series, loss of Achilles-tendon reflexes showed a statistically significant correlation with long term diabetes. In order to decide whether my own series of patients can be regarded as representative from a neurological point of view it is compared with regard to its composition and the occurrence of abnormal reflexes with other unselected series in which sufficient data on each individual patient are available. As the findings in the latter series showed good agreement with my own findings, and as the series did not reveal any evidence of false correlations, it is concluded that my series may be regarded as representative. According to these analyses it must be considered to be proved that diminished vibratory perception on the feet and abolished tendon reflexes in the legs are correlated with the duration of diabetes.

No correlation could be demonstrated between abnormal reflexes and the severity of the disease. The series did not reveal any correlation between the frequency of abnormal reflexes and age in patients up to 60 years; the youngest age group, 10–19 years, was too sparsely represented to allow definite conclusions. No sex difference as regards abnormal reflexes in the legs could be

demonstrated. Abnormal reflexes were more frequent in patients over 60 than in younger patients.

The analyses were performed on the presupposition that good or poor diabetic control if this might be of importance, was not correlated with any of the other factors.

Chapter 4

CONCLUSIVE CONSIDERATIONS ON THE PATHOGENESIS OF NEUROGENIC AFFECTION IN DIABETICS

Various statistical correlations are compatible with the hypothesis that diminished vibratory perception on the legs and the abnormal reflexes are due to diabetic angiopathy of the vasa nervorum. This hypothesis is supported by the fact that histological studies have demonstrated a close relationship between diabetic angiopathy of the vasa nervorum and objective neurological findings.

The increase in the vibratory perception threshold on the fingers seems to be a concomitant to diabetic disease since no correlations could be demonstrated In spite of this and in view of the distinct correlation between impaired vibratory sensation on the feet and the duration of diabetes, it seems most reasonable to conceive the pathogenesis as being of a metabolic nature.

Ischaemia tests have revealed that the diabetic status is of significance in the metabolism of the nerves and in vitro experiments have shown that insulin exerts an influence on the isolated sciatic nerve of the rat. The results of studies on the nerve-conduction velocity seem to suggest that metabolic factors influence the function of the peripheral nerves in diabetics. The theoretical basis for a metabolic genesis of the concomitant increase in the vibratory perception threshold is thus present.

Studies on vibratory perception during ischaemia have shown that the relation between the diabetic status and impairments in the vibratory perception is very complex. If the processes underlying the concomitant increase in the vibratory perception threshold are equally complex, this may explain why it was not possible to demonstrate any correlation with the immediate diabetic status.

Even though there is thus a theoretical basis for a peripheral metabolic genesis of the concomitant" increase in the vibratory perception threshold, disturbances in the central nervous system must also be taken into consideration after it has been shown that insulin exerts a direct action on the central nervous system.

Danish Summary

Kapitel 1

DIABETISK NEUROPATI

Der gives en kort oversigt over de hyppigste neurologiske abnormiteter som er beskrevet i litteraturen under betegnelsen »diabetisk (poly)neuritis« eller »diabetisk neuropati«.

Der er enighed om, at langt de hyppigste neurologiske manifestationer hos patienter med diabetes er lokaliseret til underextremiteterne, men der er stor uoverensstemmelse mellem de forskellige forfattere med hensyn til afgrænsning af begrebet »diabetisk neuropati«. De subjektive symptomer er tit meget vanskelige at vurdere, i de senere aar er der derfor en tendens til at indskrænke begrebet »diabetisk neuropati« til kun at omfatte patienter med objektive neurologiske fund.

Hyppigheden af »diabetisk neuropati« angives meget forskelligt, til en vis grad beroende paa kriterierne for diagnosen. Der er ogsaa uenighed om, hvorvidt »diabetisk neuropati« er korreleret til sygdommens sværhedsgrad, regulation og varighed, samt til patienternes alder. Disse uoverensstemmelser kan delvis skyldes forskellige kriterier for diagnosen, men kan ogsaa i nogle tilfælde bero paa, at der ikke er taget hensyn til muligheden for falske korrelationer betinget af materialets sammensætning. De fleste arbejder er kun sparsomt dokumenterede og resultaterne unddrager sig derfor en nærmere vurdering.

Kapitel 2

VIBRATIONSSANSEN HOS DIABETIKERE

Vibrationssansen kan undersøges med stemmegafler eller biothesiometre, som er simple elektromagnetisk drevne apparater.

Stemmegaffelundersøgelser er behæftet med en række principielle fejlkilder. Man kommer let til enten at underdiagnosticere vibrationssansaffektion hos unge, eller til at overdiagnosticere abnormiteter hos ældre mennesker, som fysiologisk kan have en høj tærskel. Resultaterne af stemmegaffelundersøgelser af diabetikere gennemgaas.

Ved biothesiometerundersøgelse maaler man direkte tærsklen for vibrationssansen, oftest arbitrært udtrykt i volt. Biothesiometerundersøgelser af diabetikere refereres. I min egen serie (STEINESS 1957b) fandtes en statistisk signifikant korrelation mellem høj vibrationstærskel paa fødderne og diabetesvarigheden. Dette fund støttes af andre arbejder men er ikke statistisk underbygget i disse. I modsætning hertil fandt MIRSKY & AL. (1953) ikke nogen korrelation til diabetesvarigheden i en stor indgaaende statistisk analyseret serie.

Paa pegefingeren er vibrationstærsklen statistisk signifikant forhøjet hos diabetikere, men det er ikke lykkedes at paavise nogen korrelationer.

Forekomsten af andre objektive neurologiske fund var ogsaa statistisk signifikant korreleret til diabetesvarigheden i mit materiale, men ikke i MIRSKY & AL.s serie. De to materialer er saaledes forskellige i neurologisk henseende.

KAPITEL 3

REFLEXABNORMITETER HOS DIABETIKERE

Forekomsten af afsvækkede og ophævede dybe reflexer er sammen med nedsat vibrationssans, almindelig anerkendt som et tidligt objektivt neurologisk fund hos patienter med diabetisk neurogen affektion. Achillesreflexerne er de første dybe reflexer som falder bort. Hyppigheden af reflexabnormiteter hos diabetikere er ikke aftaget efter insulinbehandlingens indførelse.

Forekomsten af ophævede achillesreflexer var statistisk signifikant korreleret til langvarig diabetes i mit materiale. For at afgøre, hvorvidt mit patient materiale kan betragtes som repræsentativt i neurologisk henseende sammenlignes materialets sammensætning og forekomsten af abnorme achillesreflexer med de faa, tilfældigt sammensatte serier hvor der er meddelt tilstrækkelige oplysninger om hver enkelt patient. Da fundene i disse materialer viser god overensstemmelse med mine fund, og da der ikke i nogen af materialerne findes tegn til falske korrelationer konkluderes det, at mit patientmateriale maa betragtes som repræsentativt. Efter disse undersøgelser maa det betragtes som bevist, at nedsat vibrationssans paa fødderne og ophævede dybe reflexer paa benene er korreleret til diabetesvarigheden.

Der fandtes ingen korrelation mellem reflexabnormiteter og sygdommens sværhedsgrad. I de analyserede materialer fandtes der ingen korrelation mellem hyppigheden af reflexabnormiteter og alder for patienter under 60 aar den yngste aldersklasse 10–19 aar gamle var for sparsomt repræsenteret til at tillade sikre slutninger. Der fandtes ingen kønsforskel med hensyn til reflexabnormiteter paa benene. Hos patienter over 60 aar var abnorme reflexer hyppigere end hos yngre.

Analyserne er foretaget under den forudsætning at god eller daarlig diabeteskontrol, saafremt denne maatte være af betydning ikke er korreleret til nogen af de øvrige faktorer

Kapitel 4

SAMMENFATTENDE BETRAGTNINGER OVER PATOGENESEN TIL NERVEAFFEKTION HOS DIABETIKERE

Forskellige statistiske korrelationer er forenelige med hypotesen om, at ned sat vibrationssans paa benene, ligesom reflexabnormiteterne skyldes diabetisk angiopati af vasa nervorum. Denne hypotese støttes af, at der ved histologiske undersøgelser er paavist en nær sammenhæng mellem diabetisk angiopati af vasa nervorum og objektive neurologiske fund.

Forhøjelsen af vibrationstærsklen paa fingrene synes at være en »konkomitant« til den diabetiske sygdom, idet det ikke er lykkedes at paavise nogen korrelationer Til trods herfor forekommer det, sammenholdt med den tydelige korrelation til diabetesvangheden paa fødderne mest nærliggende at opfatte patogenensen som metabolisk.

Ischæmiundersøgelser har vist, at diabetesstatus er af betydning for de diabetiske nerves metabolisme, og ved in vitro forsøg er der paavist en effekt af insulin paa den isolerede rotte ischiadicus. Resultaterne af undersøgelser over nerveledningshastigheden hos diabetikere synes at kunne tyde paa, at metaboliske faktorer ved diabetes influerer paa de perifere nervers funktion. Det teoretiske grundlag for en metabolisk genese til den »konkomitante« tærskelforhøjelse er saaledes til stede.

Undersøgelser over vibrationssansen under ischæmi har vist, at sammenhængen mellem diabetesstatus og vibrationssansabnormiteter er meget kompliceret. Saafremt det er lige saa komplicerede processer som ligger til grund for den »konkomitante« tærskelforhøjelse, har man maaske heri forklaringen paa, at det ikke er lykkedes at paavise nogen korrelation til den øjeblikkelige diabetesstatus.

Selvom der teoretisk er grundlag for en perifer metabolisk genese til den »konkomitante« tærskelforhøjelse maa en central funktionsforstyrrelse ogsaa tages i betragtning efter at det er paavist, at insulin har en direkte effekt paa centralnervesystemet.

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PLASMA PROTEIN PATTERN IN COURSE OF ACUTE INFECTIOUS DISEASE

by

SVEN BELFR

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SUPPLEMENTUM 395

FROM THE DEPARTMENT FOR INFECTIOUS DISEASES, MALMÖ GENERAL HOSPITAL, MALMÖ
HEAD: [illegible], M.D.

PLASMA PROTEIN PATTERN IN COURSE OF ACUTE INFECTIOUS DISEASE

BY

SVEN BELFRAGE

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Inflammation The local response to injury (STONER in "The Biochemical Response to Injury" ed. by STONER 1960)

Primary inflammatory blood protein reaction (ODENTHAL 1958) Changes occurring initially in the disease at the same time as the local signs of acute inflammation and in the following course modified in close connection with them. ODENTHAL assigns the increase of the α-globulins and fibrinogen, the decrease of serum albumin and the appearance of CRP in the serum to this group

The term *primary inflammatory reaction* is used here to include local signs of acute inflammation and necrosis and their general consequences, fever and leucocytosis as well as the above mentioned protein changes.

Secondary inflammatory blood protein reaction (ODENTHAL) Changes occurring in acute bacterial infection after a certain latency and developing at a slow and steady rate, most pronounced in chronic inflammatory states. The increase of γ globulin is assigned by ODENTHAL to this group

The term *secondary inflammatory reaction* is used here to include local signs of antibody formation in the form of enlargement of the lymphoid complex including lymph nodes and spleen, increase in the number of plasma cells and related cells in tissues and blood and the general consequences thereof in the form of increase of and β globulins and of antibody titers.

Lymphoid complex (YOFFEY 1960) Lymph nodes, spleen, sub-epithelial lymphoid tissue and thymus, built up of a mixed cell population with reticuloendothelial elements (macrophages) and lymphoid cells in the stricter sense of EHRICH (1956) including lymphocytes, plasma cells and their precursors.

Anamnestic reaction An antibody response elicited by unspecific means in animals with a well established potential immunity and directed to the specific antigen. The response may be brought about by injection of unrelated antigen or by induction of fever (ABRAHAM in "General Pathology" ed. by FLOREY 1962)

Adjuvant FREUND's adjuvant a water-in-oil emulsion of killed tubercle bacilli and paraffin with lanolin derivatives as emulsifier When mixed with adjuvant the antigen gives rise to a much strengthened antibody formation (FREUND 1947)

Endotoxin Components of the wall of various Gram negative bacteria, chemically lipo-polysaccharides, immunologically O antigens, pharmacologically pyrogens.

Abbreviations

Alb in the figures = Serum albumin.
Alk. phosph. in the figures = Alkaline phosphatase activity in the serum.
A.S.T in the figures = Antistreptolysin O titer
Bil. in the figures = Serum bilirubin.
cmm in the figures = cu. mm.
CRP = C-reactive protein.
Fib in the figures = Plasma fibrinogen.
g% and *mg%* in the figures and tables = gm per 100 ml and mg per 100 ml.
G P T = Glutamic puruvic acid transaminase activity in the serum.
Hp. in the figures = Serum haptoglobin.
Index nd *reduced index*, respectively see page 101
P B titer = Paul-Bunnell titer
m in the tables = Population mean of a distributi n.
n in the tables = Number of bserved individuals.
P in the tables = Significance level actually chieved by data.
r in the tables = Estimated correlati n coefficient.

after correlation coefficie t denotes $P<0.05$
** after a correlati n coefficient denotes $P<0.01$
*** after correlation coefficient den tes $P<0.001$

INTRODUCTION

The changes of the plasma protein picture in acute infection are now well known. While the changes on the whole vary with the severity of disease the relationship between those of the α and the immunoglobulins varies with the kind and duration of disease. This variation embraces a few different patterns of reaction to which the plasma protein picture of all sorts of infection can be assigned.

Determination of the plasma protein fractions yields most information when seen as a part of the entire clinical picture, and repeated determinations provide a good indicator of the course of the disease in a given case.

It appears, however that few systematic attempts have been made to follow up the protein changes together with other features of the clinical picture during the entire course of disease in a wide variety of infections.

In view of this observation and with the recently increased knowledge of the various electrophoretic serum protein fractions, it was considered worth while to chart and correlate protein changes with other manifestations of disease during the entire course of acute infectious disease and thereby possibly elucidate the pathogenesis of some of its aspects.

Chapter I

HISTORICAL

An acute infection implies functional, morphological and chemical disorders which are local and systemic. Alterations occur in the cellular picture of the circulating blood as well as in the composition of the plasma proteins. The changes in the leucocytes in acute and chronic infections have long been known (ARNETH 1904 SCHILLING 1912) It has long been the rule to measure the sedimentation rate (FÅHRÆUS 1918) Albumin and globulin have been assessed for many years. But it is only during the last two decades that it has been possible to study the serum proteins in detail, and then in the main with TISELIUS free electrophoresis described in 1937 and paper electrophoresis (CREMER & TISELIUS 1950)

In addition methods have been devised to estimate some individual protein substances, such as haptoglobin (JAYLE 1939) fibrinogen (for survey see JACOBSSON 1955) other types of glucoproteins (for survey see, WINZLER in the "Plasma Proteins ed. by PUTNAM 1960) and C-reactive protein (ANDERSON & MCCARTY 1950)

The plasma protein changes occurring in various types of acute infection and demonstrable by these methods are now largely known and have been reviewed in works by ANTWEILER by WUHRMANN & WUNDERLY and by RIVA (1957) by PUTNAM (1960) and by FURNESS (1961)

Here, the survey of our knowledge of protein disorders in acute infections will therefore be limited to a reasonable minimum

The first to publish electrophoretic data on patients with an infection was BLIX, who in 1939 reported values found for serum globulin fractions in the acute stage of severe pneumonia. He regularly found a marked increase of the α fraction. LONGSWORTH et al. (1939) also found decreased serum albumin in pneumonia and other infections. In 1946 MALMROS & BLIX reported an invariable increase in the γ fraction in a series of subacute and chronic infections with elevation of the E.S.R. In most cases the albumin was decreased and the α fraction increased, and in all the cases studied the fibrinogen content was increased. In 1948 BRUCE & ALLING studied the course of a number of cases of pneumonia and like BLIX (1939) they found no significant increase in the

γ-globulin in the beginning of the disease. In some cases, however an increase occurred a few weeks later

This established the characteristic plasma protein disturbances in pneumonia—which afterwards proved typical of all fairly severe bacterial infections—namely decreased serum albumin and markedly increased α fractions and plasma fibrinogen. An increased fraction is also often demonstrable, but then usually late in the course—an increase of the γ fraction being mainly a sign of a chronic infection.

In a certain virus infection namely hepatitis, the protein pattern was soon found to differ distinctly from that described above In 1943 GRAY & BARRON observed that, as a rule, hepatitis is accompanied by at most a slight increase of the α fraction and by a marked increase of the γ fraction. The γ fraction is increased already in the beginning of the disease (WUHRMANN & WUNDERLY 1957) Several authors have confirmed these findings. In addition, the haptoglobin belonging to the α_1 fraction (JAYLE & VALLIN 1952 NYMAN 1959) and the fibrinogen (SCHULZ 1953) both of which are usually increased in bacterial infection, are decreased, normal, or slightly increased in virus hepatitis. (See also POPPER & SCHAFFNER's monograph The Liver Structure and Function, 1957) In infectious mononucleosis, which is probably a virus infection, the protein pattern resembles that seen in hepatitis (COHN & LIDMAN 1946)

Reports on other virus infections differ In poliomyelitis ROUTH & PAUL (1951) found only moderate albumin and α-changes without any increase of the γ fraction. VON OLDERSHAUSEN et al (1953) on the other hand, found a distinct increase of the α fractions as well as of the γ fraction According to WUHRMANN & WUNDERLY (1957) poliomyelitis is most often associated with an increase of the γ fraction, but other types of virus meningitis and the exanthematous virus diseases of children mainly with a slight increase of the α-fractions.

Finally in primary atypical pneumonia the protein pattern is of the same type as in bacterial pneumonia often with an increased γ fraction (WUHRMANN & WUNDERLY 195)

A serum protein substance of particular interest is the *C reactive protein* (TILLETT & FRANCIS 1930) which is probably identical with the non-specific capsular swelling substance (LÖFSTRÖM 1944) also called acute phase protein (HEDLUND 1947) It appears early in acute bacterial infection, and it can be assessed by simple methods (ANDERSON & MCCARTY 1950) but it is not demonstrable in serum from a healthy person. In virus infections, on the other hand, CRP seems to occur less frequently

Chapter II

CHANGES IN ACUTE INFECTIOUS DISEASE

The changes of the plasma proteins in the acute infectious diseases enumerated in the foregoing chapter are the consequence of a process common to them all, namely *inflammation* with more or less evident necrosis.

The term inflammation has been defined as the local response to injury (Symp on biochemical response to injury STONER, 1960) or as a reaction of living tissue to injury or foreign agents, a reaction trying to repair the affected area (MARSHALL 1956)

In the scope of the word most authors include also the signs of antibody formation accompanying infection (MARSHALL 1956 EHRICH 1956)

This investigation is mainly concerned with the general consequences—functional, morphological and chemical—of the local process or processes which constitute inflammation in infectious disease.

It is impossible in infectious disease to separate the signs of injury from those of the first stages of the reaction. In other words, the primary evidence in the assessment of the intensity and extent *i e* the *activity* of the injury caused by infection, are the signs of inflammation and necrosis noted.

The general consequences of the local process, namely fever leucocytosis, and certain plasma protein changes are secondary signs in the follow up of the activity of injury The plasma protein changes in question are the elevation of the α-fractions and of fibrinogen as well as the appearance of CRP in the serum. They occur early in the course of inflammation. ODENTHAL (1958) calls these changes the *primary inflammatory plasma protein reaction*. The rise of the γ fraction and of antibody titers appear later in acute bacterial infection he therefore calls the latter changes *the secondary inflammatory reaction*.

THE PRIMARY INFLAMMATORY REACTION

It is known that a primary inflammatory reaction with increase of the α-globulins and the fibrinogen in the plasma and with simultaneous decrease of the albumin occurs not only in acute infections but also after cardiac infarction (WITAS 1955 LINKO & WARIS 1955) often in cancer (GOHR & LANGENBERG 1959 BÖTTIGER 1960) in rheumatic conditions (LONGSWORTH et al. 1939 MALMROS & BLIX 1946, SVARTZ & OLHAGEN 1948) after

mechanical trauma (HOCH LIGETI et al. 1953 PROBST et al. 1958) and following injection of pyrogens (HEDLUND et al. 1948, HEILMEYER 1959) thus in association with most kinds of injury CRP also occurs in the serum in all of these conditions (HEDLUND 1947) WUHRMANN (1957) who calls this pattern of plasma proteins the reaction pattern of acute inflammation, emphasizes that these conditions with the protein pattern of inflammation are most often accompanied by fever and leucocytosis, and he expresses the view that some fundamental process is responsible for all these changes in acute inflammation. Experiments with pyrogenic endotoxin have given a clue to the nature of some of these mechanisms (BENNETT JR. & BEESON 1950 EICHENBERGER et al. 1955 WOOD 1958, HEILMEYER 1959) though the mode of development of the protein disorders is still obscure

Those plasma components which change both early and regularly in inflammation have been called acute phase reactants (KELLEY 1952) To these belong certain glucoproteins in the α fractions, fibrinogen and CRP The word acute is generally used as an antonym of chronic to describe the duration of a disease. These reactants however reveal the presence of an injury whether acute or chronic. Here the term *primary inflammatory reaction* coined by ODENTHAL will be used to designate all those inflammatory changes which promptly and regularly occur in the presence of injury and thus reflect the activity of the latter In the present work the term includes also fever and leucocytosis, *i.e* a wider conception than that given by ODENTHAL who used it only for the above mentioned protein changes. All these changes are components of the primary inflammatory reaction whether the lesion is due to infection, to mechanical or chemical trauma, to ischaemia, rheumatic fever rheumatoid polyarthritis or cancer

The α glucoproteins that react promptly to injury are haptoglobin, which is a part of the α_2-fraction, and orosomucoid and SCHULTZE's α_1-antitrypsin (1962) parts of the α_1-fraction particularly the latter forms the apex of the fraction. These 3 proteins increase much in the presence of injury (JAYLE et al. 1955, SONNET 1956, LAURELL & ERIKSSON 1963) and are thus the dominant variables in the α fractions. When they increase, the α fractions *in toto* also increase—in this way then, these are also components of the primary inflammatory reaction.

THE SECONDARY INFLAMMATORY REACTION

The increase of the serum γ fraction, which is called the secondary inflammatory reaction by ODENTHAL, does not occur promptly or regularly in acute bacterial infection.

As early as 1939 TISELIUS & KABAT found antibody to be electrophoretically identical with the γ globulin component of the serum in rabbits. It is generally accepted that the main part of the γ fraction consists of antibody protein (GITLIN et al. 1959 ANKER

1960) though HUMPHREY (1960) is somewhat sceptical

It may now be regarded as established that the bulk of the γ globulin is formed in the plasma cells (BING 1940 FAGRÆUS 1948, COONS et al 1955 and NOSSAL 1959)

Immuno-electrophoresis (for survey see HEREMANS 1960) has identified 3 main components of the γ and β-fractions with the character of antibody namely γ β_{2A} β_{2M} (or γ_{G} γ_{1A} and γ_{M}) they are not separable by ordinary electrophoresis. Of the two latter minor components, β_{2M} is probably produced by lymphoid cells other than the plasma cells (WALDENSTRÖM 1944)

The changes in the tissues preceding the increase of the γ globulin and antibody formation, consist of a proliferation not only of the plasma cells but also regularly of reticuloendothelial elements, of primitive reticular cells and of lymphocytes (TALIAFERRO 1951) This explains the difficulties encountered in ascertaining which type of cells is responsible for the production of globulin. RINGERTZ & ADAMSON's (1950) immunization experiments on rabbits illustrate this point. They showed that after a subcutaneous injection of a suspension or extract of bacteria all these components of the regional lymph nodes proliferated in a certain order The plasma cell proliferation and new formation of germinal centres preceded the increase of the antibody titers in the peripheral blood A very transient phase of granulocytic increase preceded the other changes.

It appears justified to regard reticuloendothelial cells, primitive reticular cells, plasma cells and lymphocytes as a functional entity called reticuloendothelial lymphoid tissue by TALIAFERRO (1951) and simply lymphoid tissue by YOFFEY (1960) The latter term will be used here The lymphoid activity is reflected in the number as well as in the character of such cells in the tissues and in the blood. Proliferation of these cells causes enlargement of such lymphoid organs as the spleen and lymph nodes and the formation of granulomata in various chronic inflammatory states An increased lymphoid activity may or may not result in an increase of the serum γ-globulin and antibody titer

Here the term *secondary inflammatory reaction* is to be understood as including not only the rise in the serum γ fraction, but the whole complex of features indicating antibody formation The components of this reaction are links in the immunization and/or sensitization process. In the sensitized individual the antigen antibody union in its turn induces an injury with the corresponding reaction.

More important than the circulating antibody protein—in the prevention as well as in the manifestation of diseases—is probably the growth, stimulated by antigen of competent mesenchymal cells in the entire organism (BURNET 1961)

CHANGES IN THE BETA GLOBULIN

In infectious disease the total β-globulin fraction often increases and the albumin most often decreases The un

derlying mechanism of these changes is, however complex

The β-globulin can be separated into two subfractions by the method of LAURELL et al. (1956) The faster and larger fract on β contains transferrin, which in inflammation decreases si multaneously with the albumin, and small amounts of glucoprotein and sometimes of lipoprotein. The bulk of the lipoprotein in fresh serum can be recovered from the area between the β and β zones.

The main component of the electrophoretic β fraction is the β_{1C}-globulin (LAURELL & LUNDH 1962) The rela tionship between this protein and com plement factors has been elucidated by MÜLLER EBERHARD (1961) who together with NILSSON (1960) showed that the β_{1C} changes into β_{1A} in the course of antigen-antibody reactions. The same conversion into β_{1A} occurs spontaneously on storage of the serum. β_{1A} migrates electrophoretically in the β fraction and somewhat faster than transferrin. CRP likewise migrates with the β fraction in free electrophoresis, but with the fraction in semisolid (gel) medium or in paper electrophoresis (WOOD et al 1954 HEDLUND & BRATTSTEN 1956 BUSTAMENTE et al. 1957 HEREMANS 1960) WILLIAMS & GRABAR (1955) who used immuno electrophoresis, showed that antibody protein immunologically identical with that of the γ fraction is a regular component of both β-frac tions and even of the globulin.

The changes of the β fraction are no regular manifestations of any dominant variable. Transferrin which normally represents at least half of the β fraction, is as mentioned, decreased in inflammation. This decrease rarely results in a corresponding decrease of the total β fraction. In obstructive jaundice the β-lipoproteins are in creased. The β-antibody protein as well as the β_{1C} globulin are often increased in infection. Particularly the latter is responsible for the common slight increase of the β fraction. The behaviour of the β-fractions separated by LAURELL s method, in different types of infection is, however on the whole less well known.

CHANGES IN THE ALBUMIN

The amount of albumin is decreased in acute infection as in any other diseases accompanied by inflammation.

The serum albumin level is influ enced by several factors. It depends on an adequate synthesis of albumin—which is impaired in such conditions as malabsorption and liver cirrhosis—and on a normal turnover of the albu min—in nephrosis and burns the eli mination of albumin from the body is increased—and on a normal distribu tion—in oedema the amount of extra vascular albumin is increased. Finally and of possibly greatest importance the concentration of the albumin in the serum is thought to be influenced by mechanisms of colloidosmotic homeostasis (BJORNEBOE 1943 BJORNE BOE & SCHWARTZ 1959)

Most of these factors are active also in acute infection The cause of the decrease in the serum albumin in acute infection is thus too complex to

be regarded merely as part of the primary inflammatory reaction

RELATIONSHIP BETWEEN THE CHANGES IN ACUTE INFECTIOUS DISEASE

The changes in infectious disease fall mainly into two groups, one representing the primary inflammatory reaction, the other the secondary reaction. Investigations have been made to reveal the relationship between the various components of both groups.

As for the primary inflammatory reaction such a relationship has been *found to exist.* JAYLE *and co-workers* (1955—1956) stressed the close relationship in inflammation between the haptoglobin, the seromucoid and the fibrinogen. NYMAN (1959) verified the correlation in acute infection between the haptoglobin, the α_1 fraction and the fibrinogen, and in acute infection MÄRKI (195) and ODENTHAL (1958) also found a slight relationship between some reactants of protein nature and of cellular nature.

As to the serum γ-globulin value evidence of a positive correlation with other findings is meagre Not even between the level of the γ-globulin and antibody titers in individual cases of human infection has any regular positive correlation been found and only in a few infections has a correlation been shown between the γ fraction and cellular components (WUHRMANN & MÄRKI 1960) Finally the relation between the two kinds of inflammatory reaction has received but scanty attention.

PURPOSES OF INVESTIGATION

The purposes of this investigation were

1) to establish the plasma protein *changes in the course of various* acute infections,
2) to check common patterns of reaction,
3) to correlate the chronological order and the severity of various features of the clinical picture
4) to find groups of features that are connected with each other
5) to elucidate the pathogenesis of the changes found,
and
6) particularly to clarify the mechanism responsible for the correlations found between certain groups of features.

Chapter III

AUTHOR'S INVESTIGATION

PART I

MATERIAL

The material consisted of patients with various types of acute infection admitted to a Swedish department for infectious diseases, *Malmö Epidemisjukhus*. Most of the some 900 cases were collected during the years 1956—1959. When necessary the material was supplemented with cases treated elsewhere. The material is summarized in Table 1 which gives the number of admissions to the department for infectious diseases and other departments of Malmö General Hospital and the absolute and relative number of cases of various diseases studied.

The preliminary diagnosis and the limited resources available for analysis of fresh sera were the factors which mainly decided the necessary selection of cases studied out of the total material admitted to the department. A blood sample was collected from every patient in the morning after the day of admission and was sent to the laboratory together with a note of the provisional diagnosis. Most of the cases were followed with repeated tests at about one week's interval.

For practical reasons only few young infants were included. Cases that proved to be complicated by other diseases could as a rule, not be used for the investigation.

The hepatitis group studied, and particularly the mononucleosis group was fairly large because of the peculiar blood protein changes in these diseases. The material also included large groups of cases of pneumonia, representing a typical kind of acute bacterial infection, and of virus meningitis, representing a typical kind of virus infection without co-existing bacterial infection.

Small series of chronic infection—tuberculosis—of diseases secondary to streptococcal infection and of cardiac infarction as well as data from experiments with induced fever were also studied.

The representativeness of the material

The material is a largely random selection of patients admitted to the department for infectious diseases. But the patients treated in hospital generally represent more severe cases in each disease group and they are usually also relatively old patients. This does not apply to very severe diseases such as bacterial meningitis and to

Tabl 1 Author s mate ial

Disease	Admissions Malmö General Hospital 1954—58		Cases studied Dep. Inf. Dis. 1954—58		Total material	Cases forming basis of mea curves
	Dep. Inf. Dis.	Other Depts.	Isolate number	percent of admissions	Isolate number	
Bacterial p eumonia	1024	669	74	7	74	47
Bacterial meningitis	24	8	8	33	12	11
Tonsillitis	295	427	40	23	49	44
Scarlet fever	1395	—	28	2	28	23
Typhoid, paratyphoid fever	9	—	7	78	16	8
Other kind of salmonellosis	84	—	16	19	23	20
Other kind of acute enteritis	220	66	11	5	17	14
Gall stone with infection cholestasis	355	972	44	12	83	76
Pulmonary tuberculosis	54	—	5	10	12	—
Rheumatic fever	15	45	10	66	13	6
Erythema nodos	13	25	12	93	20	8
Acute gl. nephritis	21	18	5	23	16	14
Hepatitis	94	5	94	100	135	51
Mon ucleosis	150	59	116	77	147	107
Primary typical p um nia	48	8	18	38	18	14
Viral encephalo-meningitis	142	17	59	42	62	27
Influenza	339	141	30	9	30	24
Morbilli	72	—	16	22	20	14
Rubella	4	—	3	75	18	14
Stomatitis aphtosa	29	88	11	42	11	10
Toxoplasmosis	8	—	8	100	25	21
Other kinds of infecti disease	—	—	—	—	66	—
Myocardial infarction	—	—	—	—	24	16
					919	568

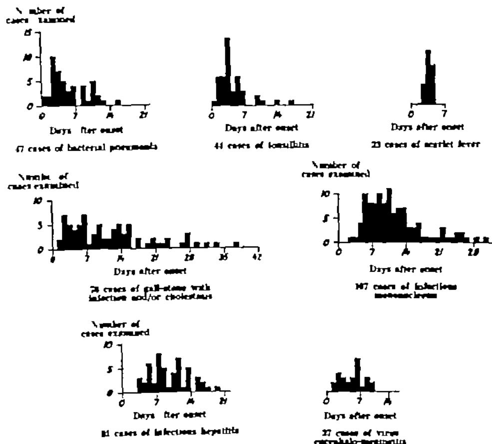


Fig. 1 Distribution of day of first serum protein examination in cases forming basis of mean curves in various groups of diseases.

contagious diseases which must, according to Swedish law be treated in the department for infectious diseases.

Most adult patients admitted to hospital because of infectious diseases were referred to the department for infectious diseases, but most of the children to the children's department. Many patients with tonsillitis were referred to the department for E.N.T and many with gall stone to the surgical department, particularly the severe cases in both groups.

In the cases studied, a varying and frequently not exactly known part of the initial course had occurred before admission. Fig. 1 gives the distribution of the day of the first electrophoretic serum examination in the larger series of diseases in the present material. It is particularly the regression of various diseases that has been studied in this investigation, and in most bacterial diseases it was not the natural course, for the course was modified by antibiotic therapy.

METHODS

The laboratory methods with their normal values and their normal ranges used in the present investigation are given in Table 2

Electrophoretic analysis After electrophoretic separation of the fractions and treatment with Bromphenol blue the filter papers were cut into segments corresponding to the separate fractions. These were eluted with sodium carbonate, and the optical densities of the solutions were read in a Bausch Lomb photometer at a wave length of 600 mμ Solutions with extinctions above 0 400—see Fig 2—were diluted to values below this limit before the results were read. The electrophoretic fractions are given as absolute values calculated from the value obtained for the total protein.

The total protein was, as a rule, determined by two methods VAN SLYKE's copper sulphate method and the biuret method The mean of both determinations was generally used. Only the copper sulphate method was used for icteric or very cloudy serum. In experiments with induced fever the ultraviolet spectrophotometric method (WADDEL 1956) was applied.

In some cases electrophoretic fraction values determined t the Central Laboratory (Head C. B Laurell) f the Malmö General Hospital w re used by the autho

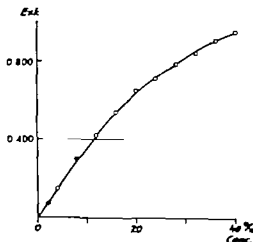


Fig 2. Relation between extinction in Bausch Lomb phot meter and concentration f alkalin bromphen l blue-soluti n t 600 m 1 cm cuvette. Only extinctions below 0.400 were used.

The normal values for the electrophoretic fractions were based on those found in samples collected before breakfast from 50 healthy ambulant, and generally young adults. The samples were generally examined the same day as they were collected. Table 3 gives the absolute and relative values obtained in these 50 persons.

RAFSKY (1952) reported a lower serum albumin and a higher γ value in normal aged, than in younger adults. In the present investigation the values in Table 3 are also used as normal in the study of groups of disease predominant in the elderly such as myocardial infarction and gall stone.

Of healthy children, aged 4—10 years a small series was examined. The mean and ranges of their values are also included in the table. It is clear that the α_2-value was much higher and the β value slightly higher in children than in adults, while the fraction was somewhat lower. OBERMAN et al. (1956) and JOSEPHSON & GYLLENSWÄRD (1957) also found the α values in children to be somewhat higher and the γ values somewhat lower than in adults but BÖTTIGER & STERKY (1962) found roughly the same α_2-values in children as in adults.

The accuracy of the electrophoretic method was calculated according to WUHRMANN & WUNDERLY (1957) on the basis of 24 determinations of one and the same normal serum and of single determinations in 50 cases (Table 4). The error of the method appeared to be smaller than the biological variation. To this error is added, in the calculation of the absolute values of the fractions, the error of the determination of the total protein.

Since the β_1 fraction decreases during storage in the frozen state because of decomposition of β_1C, β-data on sera which had been kept frozen were not used in the analysis of the material and are not included in the figures except for the series of myocardial infarction (Figs. 33 and 34). In some groups of infections no analysis of the β values found was made.

C reactive protein determination. C-reactive protein was determined in the beginning by ANDERSON—MC CARTY's method as well as by LÖFSTRÖM's (1943) method (determination of non specific capsular swelling substance). Both methods proved to give similar results in 50 cases studied (Table 5). It was therefore decided to use the former method, which is simpler and less time-consuming. In December 1957 the CRP-antiserum obtained from the suppliers (SCHIEFFELIN & Co) was found to be substantially increased in strength. Thirty CRP values in pneumonia selected at random before and after this time showed the mean value for the latter to be 4/3 of that for the former. All values noted after this time were therefore corrected accordingly.

During some periods amounts of CRP-antiserum and of the patient's serum were sucked up corresponding to 2 cm of the capillary pipette during others, 3 cm. The CRP values were corrected for this difference by increasing the values for the 2 cm group accordingly.

Table 2 *Methods used*

Methods	Author	Units		Mean	Range (± 2 S.D. or 95%)
Total serum protei	1 Copper sulphat method, PLIMMER (1944) 2 Biuret method, KINGSLEY (1940) 3 Ultraviolet spectophotometric method, WADDEL (1956)	g/100 ml serum		Ide special table 3	
Serum electrophoresis on pape	C. B LAURELL et al (1956)	g/100 ml serum		Ide special table 3	
Fibrinogen/plasma	JACOBSSON (1955) mod. LAURELL	g/100 ml plasma		0.30	0.21—0.36
Haptoglobin/serum	JAYLE (1951) mod. NYMAN (1959)	mg/100 ml serum		110	28—192
Coeruloplasmin/serum	RAVIN (1956)	Extinction units			
Protein bound hexose/serum	v HOLT (1954)	mg/100 ml serum		119	106—132
Thymol turbidity/serum	MACLAGAN (1944) slightly mod. (ph 7.8)	Extinction t 660 mμ, 1 cm cuvett Beckman B		0.04	0—0.11
Zinc turbidity/serum	KUNKEL (1947) slightly mod. (ph 7.8)	Extinction t 660 mμ, 1 cm cuvette, Bausch Lomb photometer		0.11	0.06—0.18
C-reactive protein, CRP/serum	ANDERSON & MCCARTY (1950)	mm precipit t		0	0
Erythrocyt Sedimentation R t E. S. R.	WESTERGREN (1924) JOSEPHSON & DAHLBERG (1952)	mm/one hour	male female	4 7	—11 —16
Alkal. phosphatase/serum	BUCH & BUCH (1939) slightly mod.	Buch unit	adults children		2—7 —14
Aldolase/serum	SIBLEY & LEHNINGER (1949)	unit		6	3—12
Glutamic Pyruvic Acid Transaminase G. P. T./serum	KARMEN et al (1955) mod. HANSON	Karmen unit		14	7—30
Bilirubin/serum	JENDRASSIK & GRÓF (1938)	mg/100 ml serum		0.3	0.2—1.2
White Blood Cells, W B C.	JOSEPHSON & DAHLBERG (1952)	Cells/cu.mm capillary blood	male [illegible]	5,400	2,700—8,100 2,300—7,900

			epl r 5 I os poly morph h 2 Ba j hy morphs 0.5 Lymph yt 38 M nocytes 1	33–60 0–6 0–2 15–00 0–9
Body T mperature (mea of morn-ing nd afternoo temperature)		C	...37.0°	Premen trually —37.5 ther wise—37.2°
A ti trept lysi O-titer/serum	See I I			
Pa l B nell ili r/serum				
Widal titer/serum				
C ld ggl tinin titer/serum				
T oplasma tibody titer/ser				

I figures nd t bles bbreviated g 6 mg %

Table 3 *Normal electrophoretic pattern*

Mean values of sera in 50 normal adults

Relative values	Total protein	Albumin	α_1	α_2	β_1	β_2	γ
Mean %	—	67.6	5.05	6 65	6.16	3 09	11.4
S. D	—	1.8	0.46	0 66	0.52	0.53	1.3
m ± 2 S. D	—	63.3—70.6	4 1—6 0	5.3—8.0	5.1—7.2	2.6—4 7	9.9—14.0
Absolute values							
Mean gm per 100 ml	7.04	4. 0	0.35	0 47	0 43	0.26	0.82
S. D	0.29	0.225	0.034	0.045	0 041	0.030	0 126
m ± 2 S D	6.5—7.6	4.25—5.15	0 29—0.42	0.38—0.56	0.35—0 51	0.18—0 34	0.57 –1.08

Mean values of sera of 15 normal children, 4—10 years of age

Absolute values							
Mean gm per 100 ml	7.0	4.56	0.36	0.61	0 47	0.25	0 74
S D	0.31	0.319	0.038	0.052	0.072	0.037	0 102
m ± 2 S. D	6.6—7 4	3.92—5 20	0 29—0 44	0.50—0 71	0.33—0 61	0 1 —0 32	0 53—0.94

Leucocyte counts. The number of white blood cells was usually followed at weekly intervals. As a rule, a differential count was based on 100 cells, but in mononucleosis and hepatitis usually on twice the number The atypical mononuclear cells seen particularly in mononucleosis are referred to at our hospital as "large lymphocytes with basophil cytoplasm This is a group of cells difficult to define exactly At our hospital we find in blood from normal adults 0—5 % large lymphocytes, but generally no cells with distinct basophilia of the cytoplasm.

Other methods Serological data are also given. Thus the titer of antistrep-

Table 4 *Total variation, error of electrophoretic method and biological variation on basis of single determinations of 50 normal sera and 24 determinations of one and the same normal serum*

	Total variation S. D.		Error of electrophoretic method S. D		Biological variation S. D	
	of total protein	% of fraction	% of total protein	% of fraction	% of total protein	% of fraction
Albumin	1 83	2.7	1.22	1.8	1.37	2.0
α_1	0 46	9.2	0.29	5.5	0.36	7.1
α_2	0 66	10 0	0.30	4.9	0.59	8.9
β	0 52	8 4	0.37	6.5	0.36	5 8
β	0.53	14 3	0.37	10 6	0.38	10.2
γ	1 29	11.3	0.64	5 0	1.12	9.8

Total variation based on single determinations of 50 normal sera, the error of method on 24 determinations of one and the same serum. The biological variation was calculated according to the following formula (WUHRMANN & WUNDERLY 1957)

$S.D._{Biol} = \sqrt{S.D._{Total} - S.D._{Method}}$

Table 5. *Correlation between pneumococcal capsular swelling titer and CRP-antiserum-precipitation*

Capsular swelling titer	0		2		4
1/16	1		2	7	2
			4	5	
1/4	1	2	2	1	
	2	1	1		
0	18	1	1		

CRP-precipitation (mm)

tolysin O for the Paul Bunnell reaction, the Widal reaction etc. Details are given in pertinent sections.

Many of the patients with mononucleosis or hepatitis were examined roentgenologically for hepato- and splenomegaly. The upper normal limit of the length of the spleen in adults was taken as 14 cm. Re-examination of many patients after recovery generally showed a decrease of the spleen, thus confirming that splenomegaly had existed.

In the *statistical* analysis mainly methods described by BAILEY (1959) are used.

PLASMA PROTEIN PATTERN IN THE COURSE OF VARIOUS INFECTIONS AND OF MYOCARDIAL INFARCTION

In this section various groups of infections are treated separately. The descriptions of the changes observed are, as a rule, preceded by brief historical data, a description of the material and diagnostic criteria. The findings are described and illustrated graphically by scatter diagrams and mean curves.

In the scatter diagrams the normal individual ranges are indicated by shaded areas. As a background to the mean curves the same ranges have also been inserted, although the range of variation of the mean values forming the basis of the curves is much narrower than that of individual values, the breadth varying inversely with the number of observations on which the means are based. Those mean values approaching the individual normal limit are in reality clearly abnormal.

The normal ranges apply to persons who are ambulatory. The plasma protein values are about 10 % higher for the erect position than for the recumbent (LANGE 1946, FAWCETT & WYNN 1960). The true normal zones might then have been 10 % lower than that inserted for all data on the blood proteins in the acute stage of the infections, when the patients were confined to bed.

BACTERIAL PNEUMONIA

Historical: see Chapter I.

Material. The material consisted of 47 cases of bacterial pneumonia. The cold agglutination test was negative in all. Roentgen examination invariably showed pulmonary infiltration, which regressed during the course of the disease. It was often difficult to distinguish between lobar pneumonia and bronchopneumonia because the early course was modified by antibiotic therapy, which had sometimes been started before admission of the patients. Probably about 15 of the cases were of the lobar type.

Cases of pneumonia secondary to some demonstrable basic disease or associated with some other disease capable of influencing the plasma protein pattern, such as rheumatoid arthritis, cirrhosis or leukaemia, were excluded. On the other hand, about 10 cases complicated by pleurisy and 2 by lung abscess were included. A few alcoholics without signs of liver disease were included, as were patients with emphysema.

Of the 47 patients, only 10 were fe-

males. The average age was 39 years (range 14 to 0 years)

Methods On admission and, as a rule at weekly intervals the patients were examined regarding the white blood cell picture—W B C. and the differential count—the electrophoretic serum protein pattern the E. S R. and the CRP in the serum in many cases the plasma fibrinogen and serum haptoglobin were also measured.

Findings. All of the patients initially had fever all showed CRP in the serum and in all the E. S R was more than 10 mm Almost all of the patients also had leucocytosis during fever—≥8000 leucocytes per cu. mm.

For convenience these patients are divided into one group of 26 patients in whom the temperature returned to normal within 9 days (average 5 days) and one group of 21 patients in whom the fever persisted for at lea t 10 days (average 19 days) The latter group consisted of patients who did not receive antibiotics until late or who did not respond promptly to them often patients with such complications as pleurisy or lung abscess. The mean age of the patients in the first group was 33 years and in the second group 48 years.

The cases of *pneumonia of short duration* showed marked changes in the plasma protein pattern with substantial decreases of the total protein and albumin, pronounced increases of the α fractions and the fibrinogen and a large amount of CRP in the serum (Figs. 3 and 4 a) The mean curves for these changes reached a maximum during the first week and then declined. As a rule the CRP did not disappear until some days after the fever had subsided, the α_1-curve reached the individual normal zone at roughly the same time while the α_2- and albumin curves did not return to the individual normal zone until the fourth week.

The mean curves for the β-fractions tended to rise slightly to the upper normal individual limit in the second to third week, but the γ fraction showed no definite increase in this group of patients with pneumonia.

The white blood cell picture showed a marked increase of the polymorphonuclear cells, which coincided with the peak of the temperature curve and a rapid regression simultaneous with the abatement of the fever The mononuclear cells showed no definite changes.

In the cases of *pneumonia of long duration* (Figs. 3 and 4 b) the changes were as expected much more persistent and sometimes more marked. Thus, the increase of the α-fractions and the decrease of the albumin fraction were more pronounced. The fibrinogen and the CRP increased to about the same extent in both groups. The total protein and the number of polymorphonuclear cells did not change so much as in the group with pneumonia of short duration—5 patients with prolonged pneumonia had no leucocytosis at all. The greatest difference between the two groups was, however that in the cases of prolonged pneumonia the γ fraction regularly increased. This increase occurred in the second week to reach its maximum in the 3rd to 4th week. Also the β frac

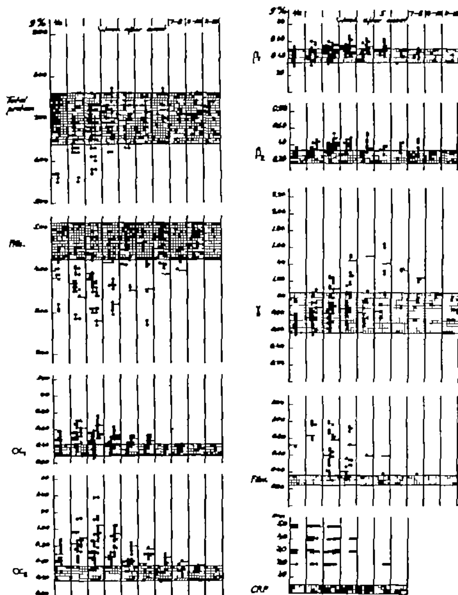


Fig. 3. *Bacterial pne monia.*

Plasma protein changes in 47 cases. Chequered zones in the diagrams represent the indi idual normal nes, the mean ±2 S D

○ 26 cases with relatively short fever < 10 days

● 21 long ≥ 10 days

tion was always increased and the β fraction more often than in the group of short duration. In the later course the increase in the γ fraction disappeared largely at the same time as the changes in the albumin and a_1 fraction or a few weeks later. This regression was slow and not always complete within two months.

Sternal marrow in pneumonia. The sternal marrow was studied in 15 patients with pneumonia. The relative numbers of different types of cells in smears was counted. The area of the parenchyma in stained section in relation to the total bone marrow was estimated. Multiplication of the above mentioned relative numbers of the different types of cells by the relative value found for the parenchyma gives a value "a cell index" expressing the number of different types of cells relati e to the entire bone marrow area. Table 6 gives the data on these 15 cases of pneumonia and on 10 controls.

Th ratio between the parenchyma and f t in the 10 normals, aged 20—60 years, agreed with Cristea finding (1949) t aut pry of about 50 / parenchyma in ternal marrow in these ages.

The table shows that in the patients with pneumonia the marrow contained more parenchyma and on the average more myeloid cells and more plasma cells than in the controls.

Fig. 5 shows a very severe case of pneumonia in a man, aged 20 with a marked increase of the myeloid, granulocytic series of cells in the bone marrow and in the peripheral blood, and with a marked increase of the

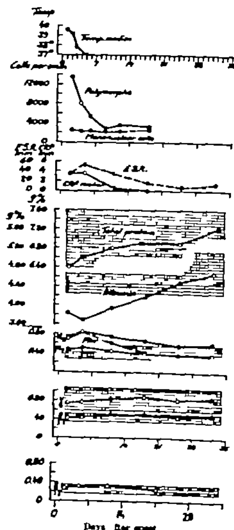


Fig. 4 a. Mean curves for changes in 26 cases of *bacterial pneumonia with fever for less than 10 day*

Chequered zones in the diagram represent the individual normal zones, the mean ± 2 S. D for different proteins. The normal zones for total protein and albumin refer to different scales. The corresponding normal one for fibrinogen, 0.24—0.36 gm/100 ml plasma, is not drawn.

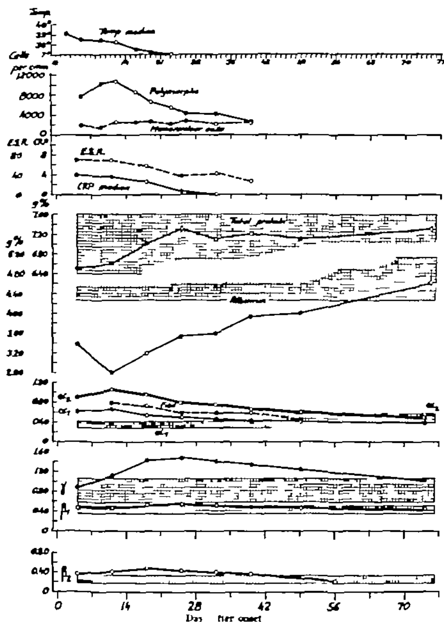


Fig 4 b Mea curves for the hanges in 21 cases of *bact rial pneumonia with fever f at least 10 day*
Chequered nes m Fig 4

Table 6. *Sternal marrow in normals and in patients with pneumonia*

	Number	Mean values and ranges		
		Relative area of parenchyma in stained section	*Cell-index = number of cells related to total bone marrow area	
			Myeloid series	Plasmacytes
Normal	10	56% (50—63)	35 (28—45)	0.4% (0.2—0.6)
Pneumonia	15	68% (40—94)	52% (28—78)	0.8% (0.2—2.8)

number of plasma cells in the bone marrow as well as the occurrence of plasma cells in the blood about the 10th day of the disease when the serum γ globulin was steeply rising to reach a high level. The γ value had 3 months later returned to normal, like other signs of disease

Comments In the cases of bacterial pneumonia of short duration the diagnosis may be regarded as firm. In the prolonged cases the diagnosis was not quite so certain. Some of the 5 patients without leucocytosis may have had atypical pneumonia even though the cold agglutination test was negative. The latter part of the curves for the prolonged cases of pneumonia is based on few cases. Despite these limitations the shape of the curves for the cases of pneumonia probably reflects the true average course of pneumonia

The curves for the cases of pneumonia of short duration are typical of a severe acute bacterial infection responding to antibiotics. The protracted cases, on the other hand in which treatment was less effective, resemble cases of pneumonia of the time before the advent of antibiotics. In the latter group the average age was rather high.

The patients with pneumonia showed a strong primary inflammatory reaction with all signs of activity pronounced and with a moderate decrease of the albumin and total proteins. Most of the protein changes persisted much longer than the fever and leucocytosis.

On the other hand, the increase in the γ fraction occurred only in patients who had had fever for at least 8—10 days, and it reached its peak some weeks after the onset of the disease. There appeared to be a correlation between the duration of distinct fever and the increase of the γ fraction. This is analysed in Chapter IV

Patients with pneumonia treated with antibiotics within one or two days of the onset and in whom the fever disappeared within a few days, had initially a strong increase in the body temperature and in the number of polymorphonuclear cells and had abundant CRP in the serum, but only a slight change in the α fractions, in the albumin and in the E. S R.

TONSILLITIS

Historical LONGSWORTH (1939) found the α fraction to be markedly increased in a case of peritonsillar abscess.

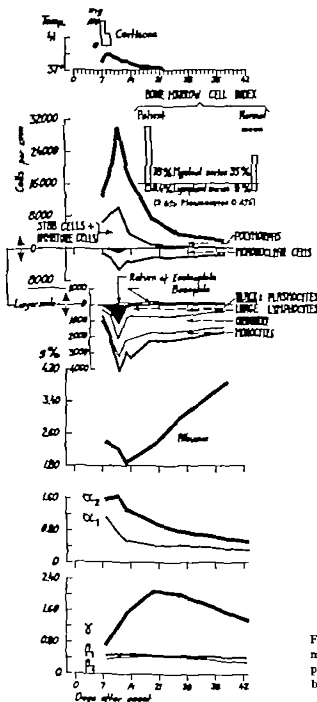


Fig. 5 A case of severe bacterial pneumonia with marked changes in the plasma proteins and in the blood and bone marrow cell picture.

MALMROS & BLIX (1946) reported an increase of the α fraction and fibrinogen, and sometimes a slight increase of the γ fraction later in the course of tonsillitis Similar findings have since been made by other investigators.

Material The series consisted of 44 mainly young patients The mean age was 24 years (range 8 years to 52 years) Thirteen of the patients had had tonsillitis within the last 2 months, usually within the last 2 weeks, so that the attack for which they were examined may be a recurrence All of them had fallen ill with a sore throat and often with high grade fever They had had symptoms on the average for two and a half days before admission Of the 44 patients, 14 had peritonsillar abscess most often requiring incision, 22 patients had exudate and 8 showed only reddening and swelling of the tonsil

In 42 of the cases throat swabs were cultured on admission and showed β-haemolytic streptococci in 9 (unsatisfactory method) The antistreptolysin O titer— A.S T in the figures and with the limiting value 210 units —was determined in 38 of the 44 cases and was found to be more than 210 units in 25 and more than 500 in 13 In at least 2/3 of the cases an influence of β-haemolytic streptococci was established, by bacteriological or serological means. Paul Bunnell s reaction was negative in all cases

Findings All of the patients had fever on admission (the average duration of fever was 6 days) all had CRP in the serum as long as the fever persisted and in all except 2 the E. S R. was more than 10 mm All except 5 of the patients examined during fever had leucocytosis

The scatter diagrams and mean curves (Figs. 6 and 7) showed changes which, like those seen in pneumonia of short duration, consisted of a relatively marked primary inflammatory reaction. The albumin concentration was, however only slightly decreased in the patients with tonsillitis, and the mean curve for the γ fraction was already initially slightly increased and rose to a level just above the upper limit of the normal individual range in the third to fourth week. The mean curves for the β-fractions, particularly β_m were likewise slightly elevated during the third week. In the patients with peritonsillar abscess the signs of inflammation were somewhat more marked than in those only with tonsillitis, and an increase of the γ fraction was somewhat more common In the cases which may have been recurrences, the 1st week values for the γ fraction and antistreptolysin O titer were on the average not higher than in the rest.

The antistreptolysin O titer was not followed regularly by repeated tests The median titer rose above the normal individual range, 210 units, already at the end of the first week of the disease, and the following weeks it was about 500

Comments Tonsillitis—sometimes recurrent—was as a rule accompanied by a marked but transient primary reaction and most often by a secondary reaction in the form of a slight parallel increase of the γ-globulin and of

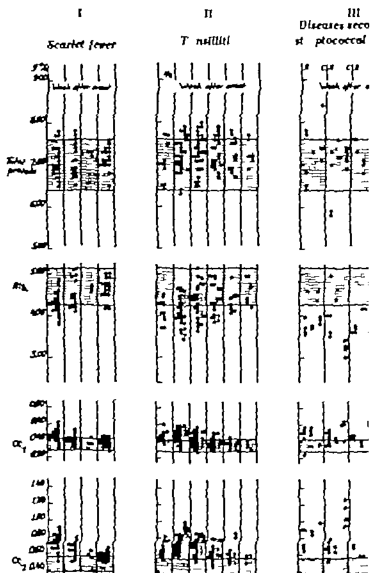


Fig 6 Pla ma p otein changes in scarlet fever tonsillitl and diseases secor
roccal infection.

Diagram I 23 cases f scarlet fever
Diagram II 44 cases of tonsillitis.
● 30 cases without peritonsillar abscess.
○ 14 cases with peritonsilla abscess
A S T alues in 14 cases in which no rise of the titer was nc

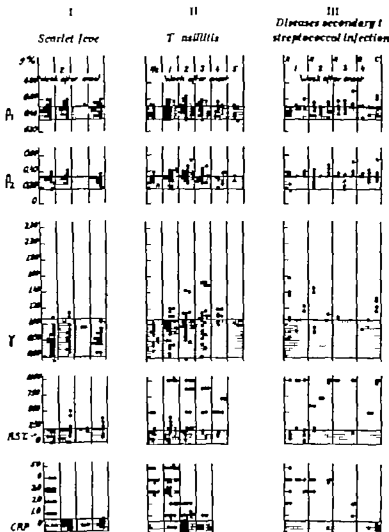


Fig 6. (Continuation.)

the antistreptolysin O titer during the month after onset.

SCARLET FEVER

Historical DOLE and co-workers (1945) followed the plasma protein changes in patients with scarlet fever (not treated with antibiotics) Some of them developed rheumatic fever They found an increase of the α fractions and sometimes an increase of the γ fraction. The changes, particularly the α_2 increase, were more persistent in those cases in which rheumatic fe ver developed. Similar findings have been reported by HARTMANN (1952) and HILLER (1952)

Material The material consisted of

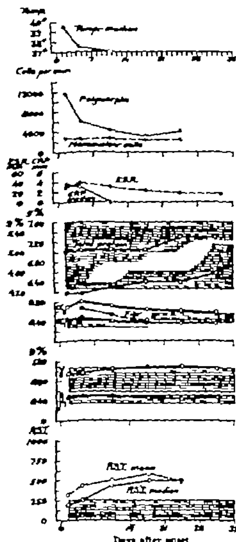


Fig. 7. Mean curves for changes in 44 cases of t...illitis. Chequered zones as in Fig. 4.

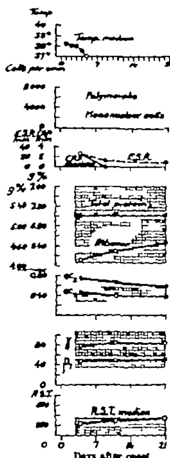


Fig. 8. Mean curves for changes in 23 cases of *scarlet fever*. Chequered zones as in Fig. 4.

23 cases of mild scarlet fever in children, aged 4 to 15 years (average 9 years). Culture of throat swabs was performed in all cases and showed β-haemolytic streptococci in 15. Penicillin treatment was started on the second to fourth day of the disease, on the average 2.5 days after onset, and the duration of fever was on the average 4 days. No complications occurred, but one patient had a recurrence after some weeks.

Methods. The patients were examined with the same method as those with tonsillitis, as a rule on the first day that penicillin was given, on the

fifth to sixth day of penicillin treatment and finally in the third to fourth week after onset

Findings. The mean curves, like the scatter diagrams (Figs. 6 and 8) show signs of a transient primary inflammatory reaction. Initially all of the patients, except one had fever the E. S R. was on the average 25 mm., but normal in 4 of the patients. The CRP was found in the serum of all except 2 Most of the patients had leucocytosis. In the second week all signs of activity of the disease had disappeared except the increase of the α_2 fraction and of the E. S R., which persisted for 3 to 4 weeks.

In these cases the albumin varied only slightly and the γ-fraction showed no increase.

The antistreptolysin O titer was not always studied. The median value did not exceed the normal individual range during the time of observation, 1 to 4 weeks from onset. Only in one case in which the disease recurred, did the titer rise to a high value during the course.

Comments In these cases of scarlet fever treated early with penicillin a distinct but brief primary reaction was seen without any definite signs of a secondary reaction in the form of a rise in the γ globulin or in the antistreptolysin O titer

DISEASES SECONDARY TO STREPTOCOCCAL INFECTION

Historical Diseases secondary to streptococcal infection are to be understood here as *inflammatory conditions which are not components but the result of prolonged or recurrent streptococcal infection.* Such secondary conditions are rheumatic fever and acute glomerulonephritis. Also some sorts of erythema nodosum have been included in this group.

Rheumatic fever In rheumatic fever LONGSWORTH et al. (1939) found the albumin to be decreased, and the α and the γ fractions to be increased. MALMROS & BLIX (1946) found the γ-fraction to be usually only very moderately increased or to border the upper normal limit SVARTZ & OLHAGEN (1948) found an increase of the γ fraction in less than half of their cases. Similar figures have been given by KROOP et al. (1954) and, for a series of children by OBERMAN et al. (1958)

In the scarlet fever series mentioned DOLE et al (1945) reported a parallel increase of the γ fraction and of the antistreptolysin O titer in some cases which developed into rheumatic fever Similar findings have also been reported by ANDERSON et al. (1948)

Erythema nodosum may be caused by a wide variety of factors (JAMES 1961) Cases with a marked increase of the antistreptolysin O titer without signs of other disease, such as tuberculosis and sarcoidosis, were assigned to this group of diseases secondary to streptococcal infection.

In a number of cases of rheumatic erythema nodosum MALMROS & BLIX (1946) found a decrease of the albumin and an increase of the α-fraction and fibrinogen, and usually a moderate increase of the γ fraction. LEINBROCK (1957) reported largely similar changes.

Acute glomerulonephritis In contrast to nephrosis and chronic nephritis, only few publications are available on the electrophoretic pattern of the serum proteins in acute nephritis. LUETSCHER (1947) found a decrease of the albumin and increase of the γ-globulin but only a slight increase of the α and β-fractions in the absence of infection. RIVA (1957) also found the increase of the α fractions to be slight in relation to the common increase of the γ fraction

Material The material consisted of 6 cases of *rheumatic fever* Three of the patients had earlier had bouts of rheumatic fever The ages ranged between 15 and 48 years (average 35 years) In all of them the rheumatic fever had been preceded 1 to 4 weeks earlier by an acute infection by tonsillitis in 5 and by enteritis in 1 In all of them the antistreptolysin O titer was elevated to at least 720 They all had joint symptoms and signs, and most of them also showed evidence of heart disease

Erythema nodosum. The series included 8 cases of rheumatic erythema nodosum. The patients ages ranged between 20 and 69 years (average 43 years) Six of the patients were females. All of them had typical erythema nodosum, in 7 cases after tonsillitis and in 1 after enteritis. The antistreptolysin O titer was elevated to at least 500 units in all.

Acute glomerulonephritis The material consisted of 14 cases. The patients ages ranged between 6 and 64 years (average 34 years) Three had had nephritis earlier In all of them an affection of the throat had preceded the onset of nephritis, which followed immediately up to 4 weeks after (average 10 days) the onset of the throat infection. The antistreptolysin O titer was increased in all of them and the lowest value noted was 210 Most of them had oedema on admission, and the more severely ill also an increase of N P N and of arterial blood pressure. Five of the cases were mild with pathological urinary findings of at most 4 weeks duration. Most of the other cases healed within some months, but in 2 of the patients chonic nephritis developed with death in uraemia 1 and 4 years, respectively later

Findings These patients with diseases secondary to streptococcal infection (Figs. 6 and 9) often showed a marked and prolonged inflammatory reaction. Especially the α_2 fraction and the E. S R. were markedly increased The fever and the increase of the polymorphonuclear cells were often less pronounced. The β and β fractions were often slightly increased. Some of the patients with nephritis had a normal β value together with a marked increase of the γ globulins. An increase in the γ fraction regularly occurred in these secondary diseases after streptococcal infection, except in a few cases of acute nephritis. The increase was usually noted at the onset of the secondary disease but was more marked during the following weeks.

In this series only cases with an increase of the antistreptolysin O titer were included The increase occurred in some already within the first week

and in all within 2 weeks after onset of the post streptococcal disease. The mean curve for the γ fraction and the median curve for the titer but not regularly the individual curves, were parallel

The total protein content which was usually decreased the first few weeks in pneumonia was normal or even sometimes slightly increased in these secondary diseases. In nephritic patients with severe oedema the total protein content was, however mark edly decreased.

No distinct parallelism could be found between the protein pattern and the severity of the rheumatic affection or the renal disease Many of the pa tients had co-existing tonsillitis and/or cervical adenitis.

Comments The cases of scarlet fever probably most of the cases of ton sillitis, and all the cases of rheumatic fever erythema nodosum and nephri tis were due to infection with A group haemolytic streptococci.

While scarlet fever and tonsillitis are manifestations of an infection, rheumatic fever and rheumatic ery thema nodosum reflect a sensitization during or after a streptococcal infec tion, and acute glomerulonephritis is probably the result of a sensitization *together* with a nephrotoxic action ex *erted by* some types of streptococci. The secondary diseases are thus caused by infection plus h perergia.

All of the patients with these diseases had a primary inflammatory reaction which in the cases of scarlet fever was distinct but thanks to anti biotic therapy short. In tonsillitis the

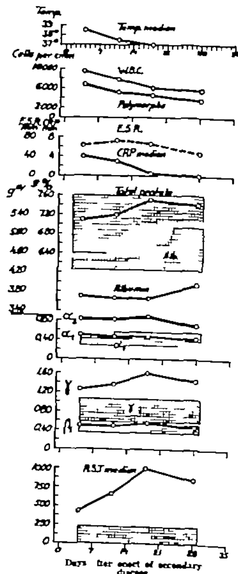


Fig. 9. Mean curves for changes in 28 *cases of disease secondary t streptococcal infecti* (acut glomerulonephritis, heumatic fever nd rheuma tic erythema nodosum) during the first month after onset of the second ary disease.

Chequered zones as in Fig. 4.

reaction was somewhat stronger and longer In the secondary diseases it was, as a rule, severe and above all long the mean curves of the secondary diseases show the course only during the first 4 weeks after the onset. Many of the cases of nephritis showed distinct signs of tonsillitis a fact which may explain why the increase of the α fractions in this series of acute nephritis was larger than that found by other authors.

In the patients with scarlet fever the γ fraction did not increase at all, but it was slightly increased in several of the cases of tonsillitis, in some of them already at the onset. The γ fraction was almost invariably increased in the secondary diseases. As mentioned, other authors have not found the γ fraction to be increased so often in these diseases secondary to streptococcal infection. The higher frequency found in the present investigation may be due to the fact that the cases were studied by paper electrophoresis, many repeatedly and the values found were given as absolute values, while most other authors used free electrophoresis and often reported only relative values.

In the cases of scarlet fever the median antistreptolysin titer was normal and reached the upper normal limit of 210 during the further course. In some cases of tonsillitis the titer was increased already in the first week—as was the γ fraction though not always in the same cases—and the median titer for the cases of tonsillitis rose moderately the following 2 to 3 weeks Of the diseases secondary to streptococcal infection and with a regular increase of the titer—owing to the criteria used in the selection of the cases—some showed normal values during the first week after onset Later nearly all the patients had high titers.

Some authors have found the antistreptolysin O titer to be regularly increased from the beginning of rheumatic fever On the other hand McCarty (1957) found the titer to rise rapidly to high values in the first week of rheumatic fever The present findings seem to be in agreement with McCarty's.

Thus a primary inflammatory reaction regularly occurred in these cases of streptococcal disease. When the reaction was relatively slight and brief no increase occurred in the γ fraction and, as a rule, not in the antistreptolysin titer either In those cases where the primary reaction was severe, and particularly when it was long as in diseases secondary to streptococcal infection, increases in the γ fraction and the antistreptolysin O titer were almost always noted. In all three groups the mean curves for γ fraction and median curves for titer were parallel.

ACUTE BACTERIAL MENINGITIS

The plasma protein picture in acute bacterial meningitis has received relatively little attention. Wuhrmann & Wunderly (1957) and Riva (1957) reported moderate changes with an increase of the α and γ globulins.

Author's material The material consisted of 11 cases of acute, bacterial meningitis. The patients ages ranged from 6 to 50 years (average 43 years)

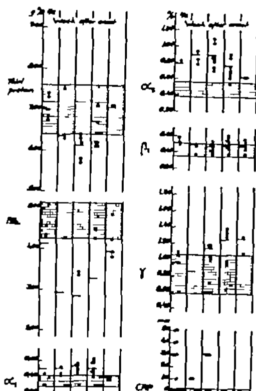


Fig. 10 *Acut bacterial meningitis.* Plasma protein hanges in 11 cases. Chequered zones as in Fig. 4.

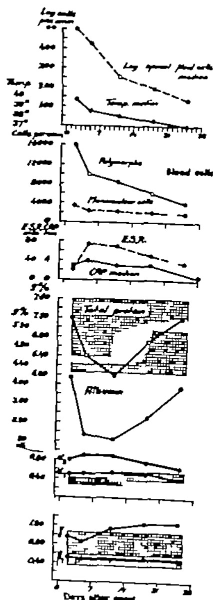


Fig. 11 Mean curves for changes in 11 cases of *acut bacterial meningitis* Chequered zones as in Fig. 4.

The bacterial agent was Meningococcus in 2 cases Staphylococcus aureus in 2 Haemophilus influenzae in 1 Pneumococcus in 1 and Listeria in 1 and in as many as 4 it was not known with certainty In all of these cases the onset had been sudden with numerous polymorphonuclear cells in the CSF and a low level of the CSF sugar All the patients were treated within a few days with antibiotics, and all of them made a recovery some promptly some after prolonged fever

Findings. The scatter diagram and the mean curves (Figs. 10 and 11) showed a sudden onset of a severe disease with high fever and increased number of polymorphonuclear cells in

the blood. Particularly in the 2 cases caused by Meningococci the number of white blood cells was very high. The blood was found to contain CRP and an increased amount of α protein. The albumin and the total protein were markedly decreased. In the beginning the γ fraction was normal but as a rule it increased moderately in the course of the disease.

Comments In these cases of meningitis the total serum protein was markedly decreased in the acute stage, particularly owing to the decreased albumin fraction. The decrease of the total protein may be partly ascribable to excessive parenteral administration of fluid but the low serum albumin is, above all, a manifestation of the severity of the acute disease.

These acute bacterial infections occurred mainly within the blood-CSF barrier but in these cases the barrier was insufficient, resulting in cellular and protein changes in the peripheral blood similar to those in the CSF (WALLENIUS 1952) The changes of the blood were of the same type and severity as in any other acute severe bacterial infection.

ACUTE ENTERITIS

A moderate increase of the α and γ fractions has been found in most cases of typhoid fever and paratyphoid fever (BENHAMOU 194 HERTEN 19 ? HUBER 1953) Enteric fever like the group of acute alimentary intoxication, on the other hand have received only little attention. HALLMAN et al. (1952) studied the changes in dyspepsia in infants and found the frac tion to be regularly increased with a maximum 3 weeks after the onset. VON STUDNITZ (1956) described a series of patients with acute alimentary staphylococcal intoxication with a slight increase of the serum α fractions and in some of them also of serum bilirubin and aldolase.

Author's material The material was not uniform. Ten cases could be distinguished from the rest of the cases of enteritis by a *markedly typhoid like* picture with fever as the dominating sign. Seven of these cases were caused by S paratyphi B one by S typhi one by S Breslau and one by S Bareilly

Of the 18 cases of Salmonella enteritis, *enteric fever* most had in the faeces S Breslau.

Fourteen other cases in this material showed the clinical picture of acute *alimentary intoxication* with an acute onset and recovery as a rule after a few days. In half of these cases culture of the faeces gave no growth of pathogenic bacteria in 4 cases it gave growth of Staphylococcus aureus and in 3 of Shigella Sonnei

Values for a further 11 cases of salmonellosis 8 of them of the typhoid type were included in certain statistical analysis (Figs. 48—46)

Methods The methods used in the other groups were employed as well as the Widal reaction with typhoid and paratyphoid B antigen, H and O

Findings. The results are given in scatter diagrams and curves (Figs. 12 and 13) The latter are based on only few data and should therefore be evaluated accordingly The high grade

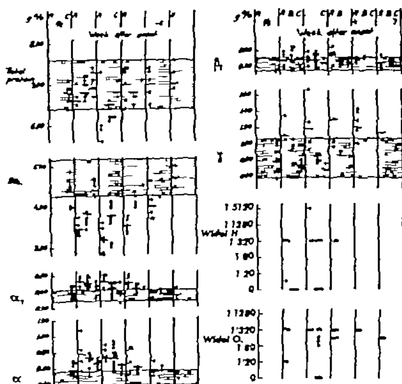


Fig. 12. Plasma p otein changes i acut enteritis
● = group A 10 cases of salmonellosis of typhoid type
• = group B 18 cases of salmonellosis of enteritis type
○ = group C. 14 cases of limentary intoxication type
Chequered zones in Fig. 3.

prolonged fever in the *cases of typhoid type* was associated with a moderate increase of the α fraction and the E S R., while the number of poly morphonuclear cells in the entire course was somewhat below normal. In 4 of the 10 patients the spleen was *palpably* enlarged. The mean albumin alue was markedly decreased, and the mean γ fraction was slightly in creased from the first week to reach a peak in the third week. In some cases, including the only case of typhoid fever the γ fraction was normal throughout the disease The Widal curve, also based on only a few data, showed an increase of the titer for both H and O antigen already in the first week. The general correlation between the γ fraction and the anti-body titers suggested by the mean cur es was not distinct in the individual cases.

The transient fever in *cases with*

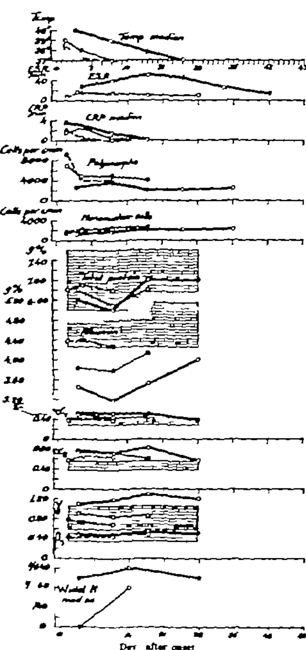


Fig. 13. Mean curves for changes in acute enteritis.
——— Mean curves in 10 cases of salmonellosis of typhoid type
——— Mean curves in 18 cases of salmonellosis of enteritis type
Mean curves in 14 cases of alimentary intoxication type
Chequered ones a in Fig. 4.

The picture of alimentary intoxication, on the other hand, was associated with a distinct increase of the number of polymorphonuclear cells but with only slight changes in the blood protein picture only a slight increase of the α_1-fraction and no change at all of the γ fraction.

Salmonella cases of enteritis type showed a blood cell picture and blood protein pattern intermediate between the typhoid and the intoxication type.

As a rule the γ fraction showed no increase but the median antibody titers were slightly increased 2—3 weeks after the onset.

Comments. The cases with predominating signs of intoxication—with or without obvious infection—manifested themselves in the form of short attacks of diarrhoea and fever with increase in the number of polymorphonuclear cells and the appearance of CRP but without any substantial change of the plasma protein fractions. The time between the ingestion of the noxious food and onset was very short.

The cases of typhoid type were due to Salmonella with a relatively slight primary injurious effect on the infected individual. The incubation time was relatively long. The spleen was often enlarged. The picture was dominated by prolonged fever with a moderate increase of the α fractions and fibrinogen, but no increase in the number of polymorphonuclear cells, and most often with an increase of the γ fraction and a positive Widal test—in some cases already from the onset of the disease.

The third group Salmonella enteritis, behaved in all respects like an intermediate form between the two others. The albumin was markedly decreased in the typhoid type of cases, but it was also usually decreased in the Salmonella enteritis group.

BILIARY DISEASE

Many investigators have studied the plasma protein pattern in biliary disease with jaundice for signs enabling differentiation between biliary disease and hepatitis. Most of them have found the electrophoretic data in this respect to be of limited value. In obstructive jaundice due to gall-stone or neoplasma, usually a larger increase of the α fraction and of the fibrinogen, but a smaller increase of the γ fraction, than in hepato-cellular jaundice (BRANTE 1952, OWEN & ROBERTSON 1956, POPPER & SCHAFFNER 1957). The fact that the haptoglobin and thereby the α_2 fraction increase relatively less in cholecystitis with jaundice than in ordinary inflammation (NYMAN 1959) explains in part the limitation of the value of electrophoresis. In this conjunction mention might be made of SELYE's experiment (1954) with rats, in which jaundice was produced by ligation of the common bile duct. — These animals reacted to an irritant with less pronounced inflammatory signs in the form of exudate-formation than control animals without jaundice.

Material. The material of biliary disease with fever and/or jaundice consisted of 83 cases, 6 of which are represented in the mean curves.

In practically all of the cases roentgen examination, operation or post mortem examination showed the underlying cause to be gall stone. None of the patients had neoplasma. Cases complicated by liver disease not related to the biliary disease were excluded. On the other hand, a few of the patients included, probably had more or less advanced biliary cirrhosis.

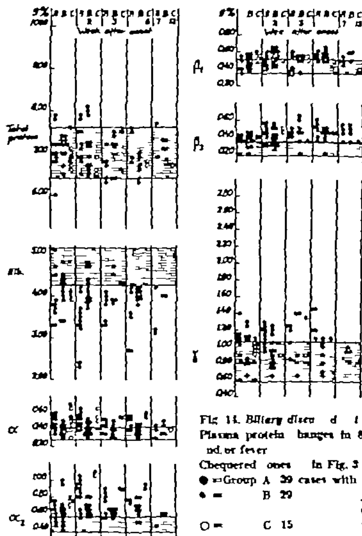


Fig. 14. *Biliary disease and gall stone*
Plasma protein changes in 83 cases with jaundice and/or fever
Chequered zones as in Fig. 3

● = Group	A	39 cases with	jaundice and fever
◆ =	B	29	jaundice, but largely afebrile
○ =	C	15	fever but largely anicteric.

The material was divided into 3 groups.

39 patients with jaundice—bilirubin in serum over 1.5 mg/100 ml.—and with a body temperature of more than 37.5 C for at least one week.

29 patients with jaundice without apparent fever

15 patients with fever without clear cut jaundice

The average age of the patients was high namely 63 years (range 23 to 85 years). The material consisted of 33 males and 50 females.

Generally speaking the biliary tract infections ran a more irregular course than the other infections described, often with recurrent bouts of sudden deterioration some of the patients did not make any recovery until after operation, and some died.

In some, the disease was probably a

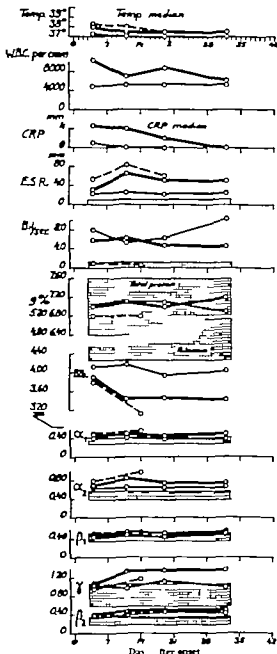


Fig. 15. Mean curves for changes in biliary disease due to gall-stone.
—— Mean curves in 33 cases with jaundice and fever
—— Mean curves [2? cases with jaundice, but largely afebrile
Mean curves in 14 cases with fever but largely anicteric.
Chequered zones [Fig 4

man festation of a chronic rather than of an acute infection.

Methods The patients were followed up with determination of serum bilirubin of alkaline phosphatase and of other enzymes, of the thymol turbi

dity in addition to electrophoretic examination which was however performed only once in most cases, and then usually in one of the first few weeks after onset of the disease (Fig 1)

Findings In the two jaundiced groups the values noted were used as a basis for the mean curves only as long as the serum bilirubin exceeded 1.5 mg/100 ml, so that the mean curves (Fig 15) for these groups do not reflect the entire course but only the jaundiced period. They show the course of the disease while it was influenced by jaundice.

The scatter diagrams and the mean curves (Figs 14 and 15) for the two febrile groups of biliary infection show a larger number of polymorphonuclear cells, a larger increase of the α fractions and of the E. S R., as well as a more pronounced decrease of the albumin, than for the afebrile group. This primary inflammatory reaction was largely equally intense in both febrile groups (insignificantly stronger in the anicteric) On the other hand, the febrile jaundiced group showed a distinctly larger increase of the γ fraction than the other two groups. In the jaundiced group without fever the mean of the β fraction was slightly increased while most of the patients of all 3 groups had an increase of their β_1 fraction.

Table 7 gives the findings in the 10 *patients with the highest fever highest serum bilirubin lowest serum bilirubin and with no fever throughout the disease* respectively The table gives the means of various data noted during the 2nd to 5th week of the disease. All data presented for a given individual refer to determinations made on one and the same day

The table shows that the mean γ fraction was markedly increased in the group with high fever and in that with intense jaundice, but not distinctly increased in the largely afebrile and largely anicteric groups. (The latter group and the intensely jaundiced group showed about the same average degree of subfebrility) This may suggest that both the temperature and the jaundice were positively correlated with the level of the γ fraction in this material of biliary disease. (See Chapter IV) As expected, the primary inflammatory reaction was strongest in the group with high fever and weakest in the group without fever while the groups with profound jaundice and without jaundice respectively occupied an intermediate position. The mean value for aldolase was found to fall within the individual normal range in all the groups, including the group with intense jaundice. The β_1-fraction was increased particularly in the intensely jaundiced patients.

What was the *morphological counterpart* of the increase in the γ value in these biliary infections? Of 10 patients with a high γ fraction value, average 1.84 g/100 ml. which were operated upon or examined post mortem, most showed marked inflammatory changes in and around the gall bladder Only one of them exhibited pronounced, and one, insignificant morphological signs of biliary cirrhosis. In most of the cases, however

Table 7 *Findings in extreme groups [illegible] biliary di case (due t gall t ne)*

4 groups highly f brile afebrile I tensely ja ndiced, ot ja ndiced.
For each group mea alues of findings in 10 cases.
For each case [illegible] data obtained on one and the same day I 2nd I 5th week of disease

Group		Temp C	Bil/s mg	Aldehyde's ntiu	E.S.R. mm	g	α_1 g	β_1 g	β_2 g	γ g	W.B.C. per cu. mm	Mea duration be tween onset nd recording eks
A. High fever ≥ 38 0°	10	38 6	8.0	10	72	0.34	0 85	0 19	0 47	1 61 (1 13—2 86)	7,300	3 4
B N fever from onset	10	37.0	7.5	13	20	0 39	0 68	0 33	0 47	1.00 (0 71—1 43)	4,600	2.8
C. I tense jaundice Bil/ ≥ 0.5 mg %	10	37 4	16 4	10	37	0.46	0.81	0.88	0.37	1.51 (1 14—2.17)	6,700	3.1
D N jaundi Bil/s < 1 5 mg %	10	37.6	0.5	8	61	0.50	0 84	0.31	0.44	1 08 (0.70—1.34)	7 100	3 0

dity in addition to electrophoretic examination which was, however performed only once in most cases, and then usually in one of the first few weeks after onset of the disease (Fig 1)

Findings In the two jaundiced groups the values noted were used as a basis for the mean curves only as long as the serum bilirubin exceeded 1.5 mg/100 ml, so that the mean curves (Fig 15) for these groups do not reflect the entire course but only the jaundiced period They show the course of the disease while it was influenced by jaundice.

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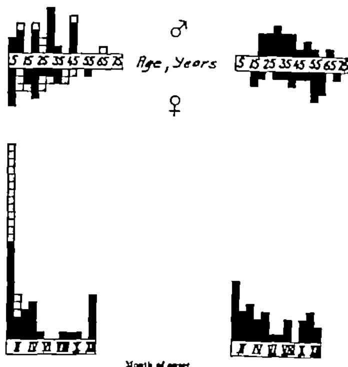


Fig 16. Age distribution and seasonal distribution of hepatitis.
Right 51 epidemic cases, 1 probably cases of infectious hepatitis.
Left 38 sporadic cases.
Solid squares cases in Malmö.
Striated squares cases in the area of Borås.

ies. These included cholecystography and bromsulphalein test. On the other hand, biopsy was done in only a few cases.

The criteria used for the cases with suspected serum hepatitis were the same except for the known administration of fibrinogen some months before the onset of the disease. The criteria for the toxic cases were *pari passu* the same.

MATERIAL

(Tables 8 a and 8 b) The *infectious hepatitis* series consisted of 6 cases belonging to the oyster-epidemic in Sweden in 1955—56 with an incubation time of about 30 days 11 cases from a gipsy-camp in the winter of 1956—57 11 cases contaminated in a nursery in Malmö in the winter of 1957—58 16 cases belonging to an epidemic in Västergötland in January—March 1959 and cared for at the hospital for infectious diseases in Borås and 6 cases belonging to other epidemics.

In addition, 46 cases belonging to an epidemic at Vipeholm Mental Hospital in 1959 were studied during the

Table 8 a. *Mean of maximal changes in various groups of hepatitis*

	Number	m Age Years	per cent cases with fever	median fever duration in febrile cases Days	m Polymorphs in blood (admission) 1000/cu. mm	m max. Bilirubin mg %	median icterus phase (Bili > 1.5 mg %) Days	m max. Thymol turbidity I.U.	median thymol positive phase (Thymol > 0.11 cal.) Days	m max. Alkal. Phosphatase U/ml
Epidemic cases of probably infectious hepatitis Children < 12 years	16	7.0 4—11	81	7.0 3—18	2.0 11 1.3—3.8	5.5 0.7—14	16 0—37	0.52 0.12—1.07 1 case normal	32 0—60	26 15—38
Patient ≥ 12 years	35	30 12—61	94	10.0 2—19	2.6 n 25 0.8—4.6	8.1 0.3—20	28 0—83	0.63 0.10—1.21 1 case normal	61 0—230	17 5—35
Sporadic cases of hepatitis	38	37 13—68	54	10.0 3—34	2.8 n 37 1.2—7.6	12 3.0—31	35 8—160	0.38 0.03—1.30 7 cases normal	45 0—270	15 6—34
Probably fibrinogen transmitted cases of serum hepatitis	12	52 22—77	42	16.0 5—30	2.1 n 11 1.5—3.3	16 1.2—34	55 0—160	0.48 0.08—0.84 1 case normal	55 0—>180	14 9—18
Probably *toxic cases*	8	53 37—73	62	13.0 3—21	4.9 8 2.4—8.3	18 5.0—36	60 20—80	0.13 0—0.34 5 cases normal		24 17—38

	Number	x̄ m min Albumin	Day median h peak phase (Alb < 100 x)	x̄ m max α1	x̄ m max α2	x̄ m max β	x̄ m max γ	x̄ m max	Day median hyperγglobulin phase > 1.10	mg% m max Haptoglobin
Epid mic cases of probably infectiou hepatitis Children < 12 years	16	1 07 3 09—4 48	19 0—49	0 41 0 37—0.48	0 61 0.51—0 75	0.54 0.47—0.63	0 42 n 0 38—0 49	1 33 0 89—2 07 1 ase norm 1	20 0—102	73 14 0—139 2 cases < 30
Patient ≥ 12 years	25	3.77 2.38—4.84	28 0—132	0 42 0 33—0.49	0 59 0.47—0 78	0.52 0 37—0 67	0.38 n 13 0.31—0.45	1.58 0 90—2.30 2 ses norm 1	54 0—230	70 22 21 133 4 cases < 30
Sporadic case of hepatitis	38	3 80 2 89—4.83	30 0—213	0 42 0.36—0.52	0.55 0 48—0.66	0.55 0.43—0 68	0.37 29 0.31—0 46	1 48 0 67—2 81 4 cases norm 1	53 0—270	37 n 20 0—118 10 cases < 30
Probably *fibrinoge trans-mitted case* of serum hepatitis	12	3.78 1 99—1 71		0.38 0 37—0 49	0.51 0 33—0 64	0 49 0.28—0 74	0.36 n 11 0.31—0 33	2.12 1 03—1.38 1 case norm 1	—	13 10 0—83 4 cases < 30
Probably *tact case*	8	3 62 2.81—4.50		0 48 0 38—0 84	0 65 0.54—0 80	0 55 0 48—0 66	0 40 6 0.35—0 48	1.21 n 7 0 85—1 47 2 ses orm 1		

Table 8 b. *Epidemic cases of infectious hepatitis—mental ho pital cases—examined 2—6 m nths after onset*

	Number	Mean	Range	Number of abnormals	
Thymol turbidity ext.	41	0.17	0.03—0.50	22	> 0.11
Zinc. turbidity ext.	41	0.22	0.08—0.39	23	> 0.18

later course of the disease but as a rule, not with electrophoresis. These patients were mentally deficient males.

Thus, 51 cases with a very probable diagnosis of infectious hepatitis were studied electrophoretically in the active stage of the disease. The ages (Fig. 16) of these patients with epidemic hepatitis ranged from 3 to 54 years mean age 22.5 years [illegible] were males 26 females. Nearly all of the cases occurred between December and April.

The 38 patients with *sporadic hepatitis* were somewhat older average 37 years (range 13 to 68 years) This group included no small children. The 21 males were predominant in the 20—30 year age class, the 17 females in the higher age classes The sporadic cases were somewhat more evenly distributed over the year than the epidemic cases

Data on 26 other sporadic cases of hepatitis are included in Tables 9 and 1

Serum hepatitis Twelve probable cases of serum hepatitis after intravenous administration of fibrinogen and described elsewhere by CRONBERG et al (19[illegible]) were examined Eight of them were known to have received fibrinogen from the same batch as at least one of the other patients with typical hepatitis Four others had fallen ill with a typical clinical picture 70—120 days after injection of fibrinogen, which was probably contaminated, other means of infection being improbable The mean age was high 5? years (range 22—69 years) Two thirds were females. The mean incubation time was 88 days.

Two young women who had received fibrinogen from the same batch had very mild hepatitis after an incubation time of only 40—60 days. Their disease may possibly have been infectious hepatitis transmitted by means of fibrinogen.

"*Toxic hepatitis* was probably present in 8 cases of liver disease with jaundice. The mean age of the patients was 52 years (range 37—73 years) Seven were preceded by medication with drugs known to be able to cause liver damage, namely chlorpromazine and iproniazid in 2 cases each and sulfadiazine sulfamerazine cinchophen and perchlorazine in 1 case each.

Immediately before the onset of "hepatitis" the eighth patient had taken piperazine, a drug not known to damage the liver The serum bilirubin was 8.8 mg/100 ml. The serum aldolase and transaminase were substantially elevated, alkaline phosphatase 18 units, thymol turbidity 0.05 the fraction was slightly elevated The disease healed Cholecystography afterwards revealed no signs of a pathologic condition. [illegible] If a year later h again took the drug.

He f ll Ill immediately with nausea, excretion of dark urine and pale faeces for some days, but he was not medically examined. Hi illnes was probably due to liver i jury by piperazine

FINDINGS

In the description of the epidemic cases of probably *infectious hepatitis* the 16 children below 12 years are dealt with separately from 35 adults. This, because of a distinct difference in the clinical picture between the two groups and because the older group can be compared with the group of sporadic hepatitis, which included no small children.

Data on these 35 adults and 16 children with infectious hepatitis are given by Table 8 a by the scatter diagram (Fig. 1) by mean curves (Fig 18) and by frequency curves (Fig. 19) which give the relative frequency of abnormal findings in each period of the course Finally Table 8 b gives data on 42 patients in the mental hospital and examined during the later course of the disease.

All the 51 patients with a diagnosis of infectious hepatitis recovered, but in one of them an 18 year old female, the disease recurred and possibly became chronic. Five years later her γ value was 1 75 g/100 ml., thymol turbidity 0.22 units, but serum bilirubin and G P T normal.

The course of the disease *in the adults* (Fig 18) was, as a rule the following During a short initial febrile stage signs of liver damage appeared with a steep increase of the aldolase and transaminase which reached a maximum already at the end of the first week. Then jaundice appeared and usually became most profound in the second week. The thymol turbidity increased in all but ? anicteric cases. The increase was most distinct in the 2nd—3rd week, and in half of the cases it persisted for more than ? months. The alkaline phosphatase was, as a rule, slightly increased during the first few weeks. The number of polymorphonuclear cells was often decreased, and about half of the cases showed relative lymphocytosis, which often persisted throughout the follow up period.

Roentgen examination showed slight enlargement of the spleen in some cases and enlargement of the liver in about half of the cases.

During the first weeks of the disease small amounts of CRP were found in the serum of most adults.

During the first month the plasma protein pattern showed a slight to moderate decrease of the albumin in most of the adults. The total protein was rarely distinctly decreased, not even during the acute phase but in many cases it exceeded the normal during the later course (Fig 1)

In about half of the adults the α_1 and α_2 fractions and the fibrinogen showed an initial, slight increase. The haptoglobin was usually normal, and occasionally subnormal.

Both β-fractions increased in about half of the adults. The β_1 increase reached its maximum after 3—6 weeks, the β_2 increase some weeks earlier

The γ fraction was nearly always increased—only in two adult patients did the value remain normal these ?

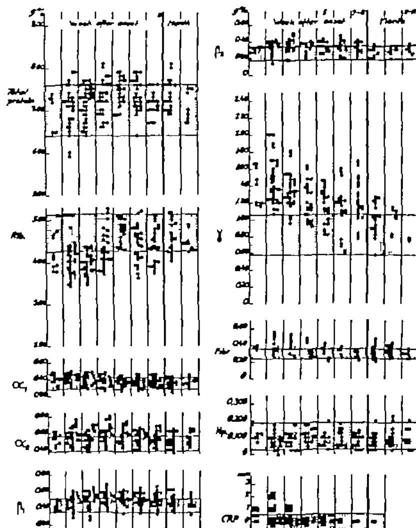


Fig 17 *Inf ct ou hepatiti*
Plasma protei hanges in 51 epidemic cases.
Chequered zones Fig 3.
● 35 cases, ges > 11 ears
○ 10 cases, ges ≤ 11 ears.

patients had a mild attack of the dis ease one without jaundice The in crease in the globulin in the other cases was noted already during the first week but as a rule reached a maxi mum in the 2nd or 3rd week. The electrophoretic γ globulin was more or less heterogenous, but usually not to the extent often seen in cirrhosis It decreased only slowly and persisted for at least ? months in about half of the cases. Judging from the persistent

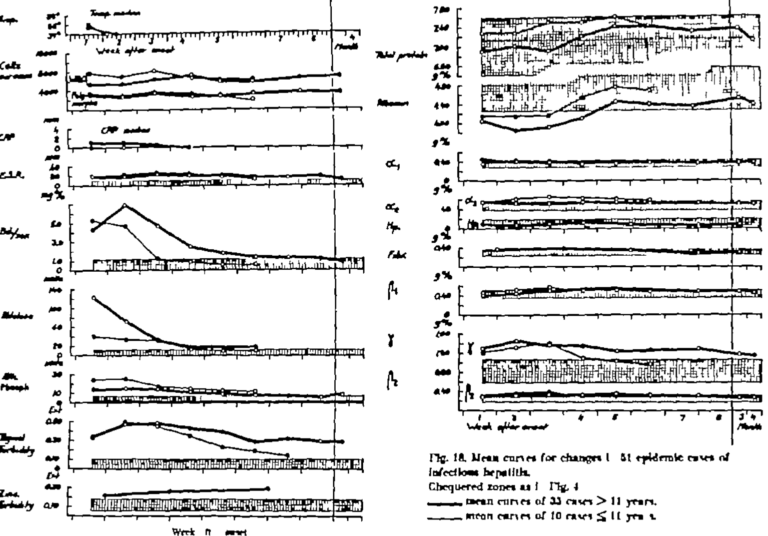


Fig. 18. Mean curves for changes i 51 epidemic cases of infectious hepatitis.
Chequered zones as i Fig. 4
—— mean curves of 33 cases > 11 years.
—— mean curves of 10 cases ≤ 11 yea s.

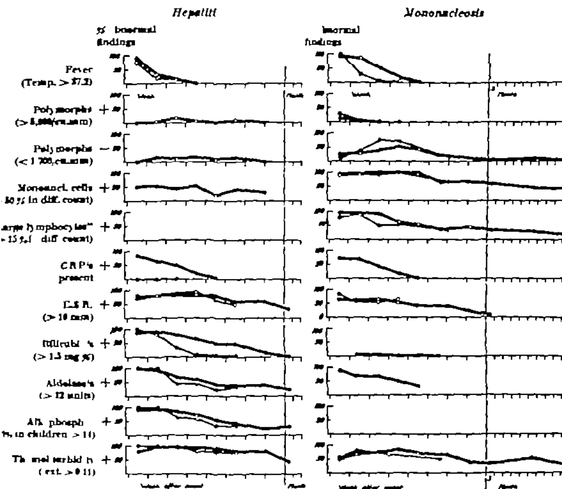


Fig. 19 *Frequency of abnormal findings in the course of infectious hepatiti and infectious mononucleosi*

In Hepatitis — Frequency curves for 35 cases, age > 11 years.
— Frequency curves for 16 cases, age ≤ 11 years.
In Mononucleosis — Frequency curves for 92 cases, age > 8 years.
— Frequency curves for 20 cases, age ≤ 8 years (electrophoretic data 1 6 cas

increase of the thymol and zinc turbidity during the second quarter In half of the patients in the mental hospital (Table 8 b) the γ fraction was increased in many of them for about half a year Re-examination after 1—3 years showed a distinct rise in some cases but in most cases the γ fraction was normal.

The course of hepatitis *in the children* was short and mild (Table 8 a) Usually the α fractions as well as the haptoglobin and fibrinogen were normal, the CRP absent in the serum and

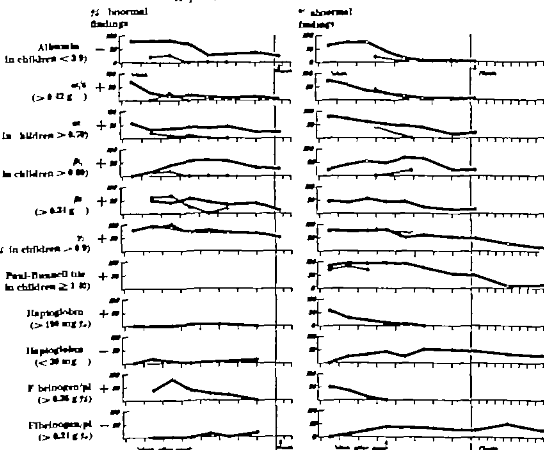


Fig. 19 (continuation)

the albumin not decreased. A few of the children had a subnormal amount of haptoglobin.

Only the γ fraction was regularly increased, usually markedly like the thymol turbidity and as in adults, both disorders persisted a long time in many of the children. The β_1-fraction was increased in half of them like in adults.

Probably serum hepatitis (Table 8 a). Two young women had a very mild and short disease (vide page 52). Several elderly patients had a severe or prolonged disease—the mean age was much higher than in the group of adults with infectious hepatitis. All in the group except one recovered. One man, 69 years old, died within a few days of onset of an acute liver failure and haemorrhages.

In this patient, who had undergone extirpation of a rectal carcinoma, the postoperati course was smooth until the 80th day after he had been gi en fibrinogen from the same batch two the patients

who also developed hepatitis. He had fever the first few days, with a maximum of 39.0 C, serum bilirubin 11.0 mg./100 ml., G.P.T. 2918 units, thymol turbidity extinction 0.21, E.S.R. 5 mm, plasma protrombin low, electrophoresis albumin 3.7, α_1 0.29, α_2 0.32, β 0.25, β 0.21 and γ 1.3 gm./100 ml., total protein 6.0 gm./100 ml., thus, only slightly elevated γ and normal subnormal α- and β-values. He became comatous and died on the 5th day of the disease. Post mortem: Extensive inflammation and necrosis of the liver with gross haemorrhages there and elsewhere.

Seven of the patients with probably serum hepatitis were afebrile during the initial course. On the average, the jaundice was more intense and the icteric phase more prolonged than in the epidemic cases. The W.B.C. value, the α, α_2 and β levels as well as the haptoglobin were somewhat lower. The thymol turbidity value was, on the average, lower than in the adult cases of infectious hepatitis, but only in one case with slight jaundice was the thymol test normal. The mean γ fraction value was distinctly higher than in the other groups. Some elderly patients with severe hepatitis showed a very high γ fraction.

Probably toxic cases (Table 8 a.) All the 8 patients gradually recovered. Three of them were operated upon. Most of them had fever. The jaundice was, on the average, just as pronounced and the icteric phase just as prolonged, as in the serum hepatitis. The alkaline phosphatase value was distinctly elevated in all of them. The chlorpromazine cases had eosinophilia. There was no distinct leucopenia and no relative lymphocytosis. The mean W.B.C., α, α_2 and plasma fibrinogen values were higher than in the other groups. Above all the thymol reaction was weaker—in 5 of the 8 cases it was normal—and the increase of the γ fraction was less distinct—in 3 of the cases the γ fraction was normal and only in one patient with rheumatoid arthritis was the γ fraction above 1.5 gm./100 ml.

Those patients, who had received chlorpromazine, perphenazine and sulfonamides, showed a picture corresponding to the type which POPPER & SCHAFFNER (1959) call the cholestatic. Biopsy in the most intensely jaundiced patient in this group showed severe cholestasis. Also those patients in whom the disease developed after treatment with iproniazid, with liver injury evidently of the necrotizing inflammatory type (POPPER & SCHAFFNER) showed a clinical picture rather similar to that of the cholestatic type, but with a high transaminase value. Operation and biopsy in one intensely jaundiced patient revealed widespread liver necrosis.

Sporadic cases of hepatitis (Table 8 a). The sporadic cases were slightly more protracted than the epidemic cases. Of the 38 patients in the sporadic group all recovered except 2 middle-aged women in whom the disease recurred and later became chronic.

About half of the 38 sporadic cases were not accompanied by fever. Jaundice was, on the average, somewhat more intense than in the epidemic cases. The haptoglobin was sub-normal in about half of them.

The thymol turbidity test was normal in as many as 7 of the sporadic

cases against only one of the adult epidemic cases, which was also an icteric

The electrophoretic blood protein pattern in the sporadic cases agreed with that in the epidemic cases except that the α_2 fraction was somewhat lower corresponding to the low haptoglobin value.

Of th further 26 sporadic cases of un known etiology incl ided in Tables 9 and 12 one was fatal. A 34 years old man died in acute fulminating hepatitis with gross bleedings after a course f one month with gradually increasing jaundice and coma. No fever E. S R. 5 mm. Plasma fibrinogen 0 15 gm./100 ml., G P T 439 units. Thymol turbidity tinction normal, 0 08 units. Serum protein alb min 2.9 α_1 0 30 α_2 0.29, β 0 42, β 0 48, γ 2.1 gm./100 ml., total protein 6 7 gm./100 ml i.e a low album and α_2 high γ- alue.

COMMENTS

This hepatitis material consists of relatively many cases of very probably infectious hepatitis—one group of children and one group of adults—a small number of very probably serum hepatitis and toxic "hepatitis" respectively and finally a larger group of sporadic cases of "hepatitis" of unknown origin.

Infectious hepatitis is thought to be an infection most common in children, in whom it usually produces no jaundice The 15 jaundiced children with hepatitis in the present material thus represent a selection of more severe cases in children but even in these the course was fairly mild. Except for jaundice of a few weeks duration, the most remarkable finding was usually a distinct and fairly persistent elevation of the γ fraction and of thymol turbidity values. The alkaline phosphatase is normally higher in children than in adults, and the relative increase in the values was largely the same.

The picture in the adult cases of infectious hepatitis was more distinct and the disease more prolonged Almost all of them fell ill with fever of varying severity and sometimes elicited by a chill. In the 6 cases of oyster hepatitis the onset was of the latter type. This is in accordance with findings in the same epidemic in Gothenburg (LINDBERG BROMAN 1956) In the other epidemics the initial fever was often slight. The following clinical course in the adults generally coincided with that reported by HAVENS JR. (1948 and 196?) and in the children with that reported by KRUGMAN et al. (1962)

Compared with the adult cases of infectious hepatitis the 12 cases of probably *serum hepatitis* were more often afebrile during the whole course, but usually somewhat more intensely jaundiced and for a longer time. They showed a slightly weaker primary inflammatory reaction including all its components, but most of them a pronounced γ-elevation. *The thymol turbidity reaction was almost always positive in both groups*

These characteristics of the serum group are largely in accordance with the findings of earlier authors (NEEFE 1946 GREEN 1950) Findings in common are the frequent insidious afebrile onset and the somewhat more prolonged course together with the very long incubation time, but not the nearly

regular occurrence of an elevated thymol turbidity value in the author's series. NEEFE, for example, found a normal or almost normal thymol reaction in 4 of 6 voluntary cases. On the other hand in an investigation in progress on cross-country runner's disease very probably a form of serum hepatitis, ZETTERBERG et al. (1951) found an elevated thymol value in almost all the cases.

The small differences found in the clinical picture of infectious hepatitis and serum hepatitis in the present material may however partly be a function of age The mean age was decidedly higher in the latter group and *age* is, on the whole *a very important determinant of the picture and the course of hepatitis* in the non immune individual (See page 133)

The 8 probably *toxic cases* usually had profound jaundice and a relatively marked primary inflammatory reaction. They differed in particular from both the infectious and the serum cases in that *they most often had a normal thymol turbidity* value and many of them also a normal value All this holds for toxic cases of the cholestatic as well as of the necrotizing inflammatory types of POPPER—SCHAFFNER.

The aetiology of the *sporadic cases* is obscure This group must surely include many cases of infectious hepatitis The age incidence and the clinical picture however fitted in somewhat better with the toxic and serum hepatitis cases.

Some of the sporadic cases particularly those with a normal thymol turbidity value may have been cases of toxic hepatitis, though of unknown aetiology Some of the patients may possibly have had serum hepatitis transmitted in association with some sort of skin traumata other than medical treatment, for example shaving under less hygienic conditions or perhaps needle pricks—several of the sporadic cases were seen in young men in the low socio-economic classes and a couple seen in seamstresses from the same working place At the after-examination nearly all of them were healthy—the possibility of their "hepatitis being the first manifestation of cirrhosis is only slight

PATHOGENESIS

The sudden onset of overt liver injury usually heralded by a febrile phase, is probably a manifestation of a sensitization The long incubation time in hepatitis as well as the increase of the γ fraction, noted already the first days of disease, is compatible with such an explanation

When largely the same clinical and pathological picture is seen also in cases of toxic "hepatitis it may be explained by an allergic reaction acting also in them On the other hand, toxic hepatitis is often associated with a normal γ fraction and thymol value This may be a consequence of the fact that only one substance not that complex of many antigens implied in an infection is the cause of the condition.

PROGNOSIS

The prolonged increase of the γ globulin in this virus infection suggests a more prolonged and profound effect on the organism than that caused by

Table 9 *Prognosi f case f h pat ii with extr me values f r serum gamma gl bulin bilirubin kldolase (r G P T) and albumin in the 1 t month f disease*

Group	Number of cases	m Age	Males	m Bilirubin/s mg	m Thymol turbidity 1 st	m γ, g%	Median weeks at hospital	N mb of u f orable cases	N mb of male lem f orable cases
I γ/ ≥1 80 g/100 ml	27 (7+7)	42	48	15	0 67	2 31	0 (+1 death)	7	1
II. γ/ <1 10 g/100 ml	12 (2+2)	36	75	13	0 00	0 90	6	0	—
III B lirubin/ ≥15.0 mg/100 ml	25 (3+5)	45	40	21	0 42	1 80	10	3 (also with high γ value)	1
IV B lirul in/ <3.0 mg/100 ml	12 (8+2)	21	33	2	0 38	1 31	1	0	—
V Aldolase/ ≥120 unit (or G P T./s ≥1000 unit)	18 (7+1)	31	63	11	0 44	1.28	5 (+1 death)	1	1
VI. Aldolase/s <27 nit (G P T./ <100 unit)	13 (5+0)	32	46	9	0 46	1.59	6	0	—
VII. Albumin/s <3.15 g/100 ml (m 2.79)	12 (3+2)	49	13	18	0 51	2 17	10	2 (lso with high γ v l)	1
VIII Albumin/s ≥4 30 g/100 ml (m 4 48)	14 (4+3)	28	71	8	0.31	1.33	3	0	—

Values in bracket Number of cases of probably infectiou hepatiti +n mber of cases of probably serum hepatitis.

an acute bacterial infection Age is, as mentioned, an important prognostic factor Can the prognosis be judged from the clinical picture in the acute stage?

The hepatitis series included 135 cases. In an attempt to elucidate the prognosis, of these patients 8 groups were selected who, during the first month of the disease, showed pronounced and insignificant changes, respectively in the serum γ fraction serum albumin serum bilirubin and level of enzymes, aldolase or G P T (Table 9)

The γ fraction was initially above 1.8 gm./100 ml. in 7 out of 9 cases with a less favourable course i e recurring chronic, or in one case fatal In only one of these 7 cases was the hepatitis of verified infectious type. Of the total series 14 women had a γ fraction of at least 1.8 gm./100 ml. In 6 of these female cases the course was less favourable while of 13 males with a high γ fraction, the course was unfavourable in only one. That man, however died. *A very high γ fraction in the acute stage thus seems to indicate the risk of recurrences and chronicity in women but not to the same degree in males*

Out of 7 patients with high γ value in the series of infectious hepatitis, as many as 4 belonged to the same family a man and 3 grown up children with value 1.8—2.3 gm/100 ml All of them recovered.

All the cases with a normal fraction and usually also normal thymol turbidity value had an uneventful but sometimes protracted course Many of them were toxic cases

A high serum bilirubin value indicated a prolonged disease, as reflected by long hospitalization, but development into chronic disease occurred in only 3 cases in which the γ fraction was also high Only slight jaundice was most often seen in young people with infectious hepatitis of short duration.

A high level of the enzyme value—aldolase or transaminase—during the first month of disease appeared to be of minor prognostic importance. The highest enzyme value was, however noted in the rapidly fatal case of serum hepatitis. Otherwise the patients with a high value often also had a high fever initially A low enzyme value in the acute stage was often noted in children with slight hepatitis but also sometimes in elderly patients in whom liver cirrhosis was primarily suspected. *The persistent increase of the enzyme level* on the other hand—even a slight increase—*reflected a persistent activity of the disease* All the 9 patients in whom the course was less favourable showed a prolonged or recurrent increase of the enzyme.

A low serum albumin suggested a prolonged and often severe disease often with profound jaundice and/or a high γ level Two cases, also with both the latter features ran a less favourable course. A normal albumin value was most often seen in children and young men with slight jaundice

Fatal cases. Of the 9 fatal cases of hepatitis admitted 1950—1963 5 ran a rapid course ending within 1 or 2 months with death in acute or subacute liver atrophy The remaining 4 patients died after 1—6

years from cirrhosis. Three of the former and all the latter w re females. The mean age was 50 years (range 20—77 years)

In the early clinical picture during the first month f disease the following char acteri tics were noted in these f tal cases. *All 5 acute o subacut fatal cas s had a low plasma proth mbin and a low no mal E S R some of them had gross haemor rhages They had a low total protein.* The 2 patient studied electrophoretically had fe fo a few days and some CRP in the serum, but a normal α fraction and a subnormal α_2 fraction as well a decreased haptoglobin and fibrinogen, in other words a ery defective primary inflammatory re action but they had markedly ele ted transaminase values. *The γ-fraction and the thymol turbidity values were not regularly high in these acute fatal cases* The findings re in accord with other reports (STAUB 1947 VAN DOMMELEN et al. 1959)

In the 4 fatal chronic cases all in femal es on the other hand the thymol turbi dity values were markedly and pe sistent ly levated from the beginning

INFECTIOUS MONONUCLEOSIS

Infectious mononucleosis is character ized by a marked proliferation of the lymphoid tissues and slight changes of the liver parenchyma. COHN & LIDMAN (1946) found the plasma protein pat tern in mononucleosis to resemble that seen in hepatitis, with a marked in crease of the γ fraction. STERLING ((1949) confirmed this and expressed the view that the hepatic lesion in mononucleosis was responsible for this increase. BEYREDER & RETTENBACHER DAUBNER (1953) like SULLIVAN et al. (1957) believe the increase in the γ fraction to be a component of the ge neral lymphoid reaction in mononucleosis SULLIVAN's series of 27 cases is the largest on record studied electrophoretically YAMASAKI s (1956) series of 12 volunteers inoculated with a Japanese form of mononucle osis showed an early and fairly per sistent increase of the γ fraction as the most conspicuous serum protein change an increase that was positively correlated with the Paul Bunnel liter

SCHULZ (1953) determined the fi brinogen in 3 cases of mononucleosis and found it to be largely normal. Otherwise no data on the fibrinogen in mononucleosis have been published, except that in the author's (1958) and NYMAN's (1959) investigations in both of which the fibrinogen level was found to be relatively low in this disease. NYMAN (1959) has shown that also the serum haptoglobin is strik ingly low and often missing in mononucleosis

Diagnostic criteria used All of the patients had a transient disease gener ally with enlargement of the spleen and lymph nodes and with the blood picture dominated by mononuclear cells including many large round cells of Downey McKinlay type. As a rule, the diagnosis required at least 50 % mononuclear cells in the differential count and at least 15 % "large lym phocytes many with a basophil cytoplasm, and a positive Paul Bunnell re action (P B reaction) with limiting titer 1/80 after absorption according to DAVIDSOHN Cases which had a neg ative P B reaction, but otherwise sat isfied the criteria and, in addition, showed an absolute lymphocytosis with more than 4 000 mononuclear

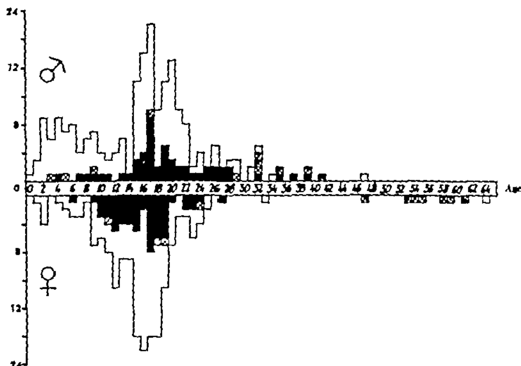


Fig. 20. Age and sex distribution of cases of *infectios mononucleosis*, studied electrophoretically against background of all 424 cases of mononucleosis admitted t Malmö General Hospital during the years 1954—1960.
Solid squares 107 cases with positive P B reaction and studied electrophoretically.
Crossed squares 22 cases with negativ P-B reaction and studied electrophoretically.
Of the latter those below 20 years had typical tonsillar changes
those above 20 years had demonstrable tonsillar changes.

cells per cu mm. were however included as a special group. Furthermore when typical throat changes were also missing negative serological tests were required to exclude the possibility of active toxoplasmosis.

MATERIAL

The scatter diagrams and the mean curves are based on 107 cases with a positive P B reaction ($\gtrsim$ 1/80). The age and sex distribution of these 107 patients is given in Fig 20. The median age of these 107 patients was 17 years. The sex distribution was roughly equal.

Fig 20 also shows 22 cases with a negative P B reaction. Of these patients 15 had no demonstrable tonsillar changes and were relatively old with the median age of 40 years (range 24—61 years). Especially many

of the women were old. In several of this group the P B titer was 0

Seven of the patients with a negative P B reaction had typical throat changes. They were younger with a median age of 11 years (range 3—19 years) Nearly all of them had the titer 1/40

Infectious mononucleosis, particularly cases with a negative P B. reaction with and without typical throat changes, was the subject of a previous paper (BELFRAGE 1962) Cases, mostly infantile, with typical throat changes and with a weak rather than negative reaction, probably represent the same diseases as true infection mononucleosis. In children a titer of 1/40 should be considered positive On the other hand, the group of usually older patients without throat changes and often without any heterophil antibodies in their serum may represent some other disease

The frequency curves (Fig. 19) give the relative number of abnormal findings regarding each detail and each period They are based on P B positive cases in this series, but for the children below 9 years of age, with a titer of 1/40 or more on 6 cases from this series and 14 further cases in which the serum protein was not examined.

In the χ-analysis of mononucleosis, data from all together 270 cases of P B positive mononucleosis were used (Table 20) For comparison, 59 cases of P B negative mononucleosis were also studied.

FINDINGS

The illustrations (Figs. 19 21 and 22) give the following picture of *P B positive mononucleosis*. Practically all the patients had fever on the average 2—3 weeks. They also had typical changes in the mononuclear blood cell picture which were most pronounced during the fever In addition, the number of polymorphonuclear blood cells was reduced, most markedly during the first afebrile weeks.

In about half of the cases a relative lymphocytosis with more than 50 % mononuclear cells persisted for 3 months, in some cases for more than a year This relative lymphocytosis was due to an increased number of mononuclear cells and a simultaneously reduced number of polymorphonuclear cells.

In only about half of the patients was the E. S. R increased, only slightly as a rule, though sometimes considerably In some of the patients the E. S. R. was normal despite severe throat changes and fever

CRP was demonstrated in the serum in about two thirds of the cases during the acute phase

The thymol turbidity was increased in half of the cases initially and in about 80 % of the cases 2—3 weeks after onset of the disease. The increase was not so marked as in hepatitis. It was largest in mononucleosis with associated jaundice. The increase persisted for a long time, sometimes for more than one year

Aldolase was increased in almost all cases. The increase was slight and lasted for only a few weeks.

The serum albumin was slightly decreased during the first few weeks, while the total protein was, if anything, somewhat increased, particularly after the first few weeks of the

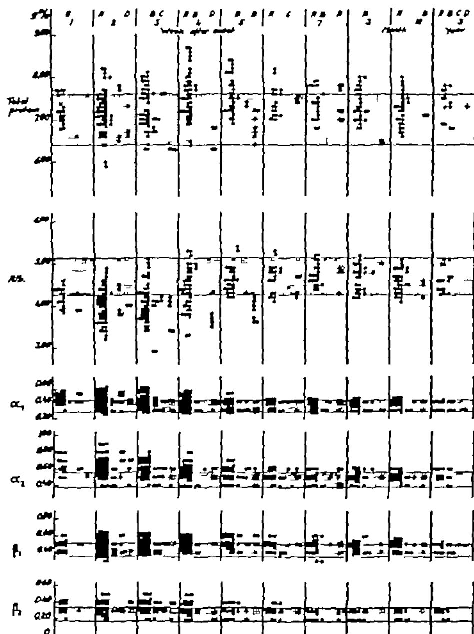


Fig. 21 *Infectious mononucleosis (and similar di eas).*

Pla ma prote hanges in 107 cases with positi and 22 cases with negati P. B. reaction. Chequered zones as in Fig. 3.

● Cases w th P. B. reaction ≥ 1/80 { Group A 85 ca es with typical ton illar changes. Group B 10 cases without typical tonsillar changes.

• < 1/80 Group C cases with typical tonsilla ha ges.

○ < 1/80 Group D 15 cases without typical t sillar hanges.

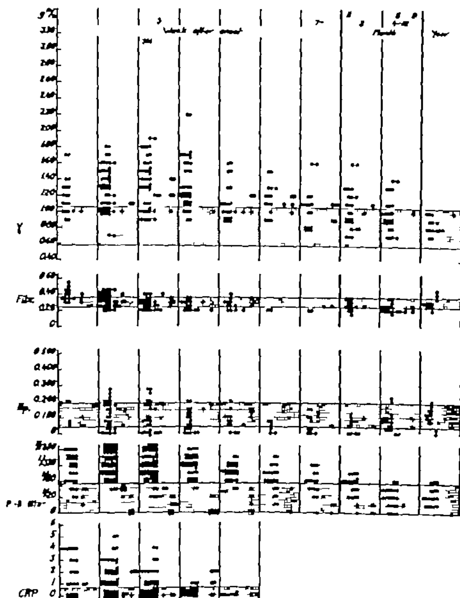


Fig. 21 (continuation).

disease, as in patients with hepatitis. The α_1 and α_2 fractions were slightly or moderately increased for the first few weeks, but this increase regressed during the following 3—6 weeks. In some cases the α_2 fraction was normal throughout, and then subnormal or no haptoglobin was found in the serum. The *haptoglobin* increased initially in only about half of the cases. This also

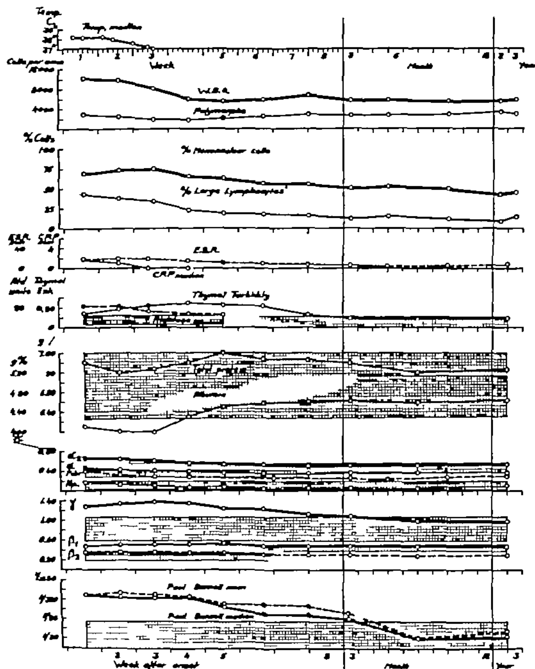


Fig 22 Mean urves for changes—absolute alues—in 107 cases of *infection mononuc leosi* with P B t ter ≥ 1/80
Chequered ones   in Fig. 4

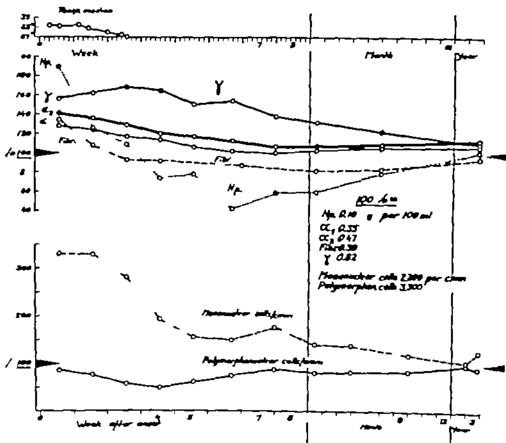


Fig 22 b Mean curves for hanges, relati to normal alues, in 107 cases of *infectious m nonucleosis* with P B titer ≥ 1/80

holds for *fibrinogen* These increases soon disappeared, and in the later course the haptoglobin level and fi brinogen level were subnormal in half of the patients. In many cases the haptoglobin disappeared completely while the change of the fibrinogen consisted of only a slight decrease. In the mean curves for haptoglobin and fibrinogen minima occurred as late as 1—2 months after the onset. In some cases the haptoglobin and the fi brinogen were subnormal for more than one year

The β fraction was slightly increa sed in about half of the cases with a maximum after one month as in hepatitis.

The β fraction also increased slight ly in about half of the cases. This increase occurred earlier than that of the β fraction and at the same time as the more pronounced increase of the γ fraction.

The γ globulin was increased initially in about 80 % of the cases with a maximum 2—3 weeks after the onset of the disease. The electrophoretic γ globulin was heterogenous, but the γ band on the paper strip was usually well demarcated from the β_2-fraction. The increase of the γ fraction persisted in about half of the cases for about 3 months and occasionally for 2—3 years.

The Paul Bunnell reaction was positive in almost all cases already from the beginning and the curve for this reaction afterwards ran parallel to the γ fraction curve a few cases were weakly positive even 2—3 years after onset of the disease.

The frequency curves for *the infantile cases* (Fig 19) showed roughly the same course as in the adults in the acute stage, but after the first week the relative number of children with fever with decreased albumin and increased α_2-fraction, rapidly diminished Thus, as a rule, the primary inflammatory reaction was more transient than in the adults. The plasma proteins in the infantile cases were not followed up long enough to allow of any evaluation of the duration of the increase in the γ fraction, but judging from the thymol turbidity test it regressed at the same slow rate as in the adults.

Findings in P B negative cases The scatter diagrams in Fig. 21 show the protein changes in the 10 P B positive cases as well as in 22 negative cases.

Most of the patients with negative P B reaction and without typical throat changes had a long and severe disease. This was reflected by a marked and prolonged decrease of the serum albumin. But otherwise the protein pattern during the course of the P B negative cases—with and without throat changes—was largely the same as in the positive cases. This seems to hold also for the changes in fibrinogen and haptoglobin.

Cases with a large γ-fraction The 16 P B positive cases with mononucleosis in the material with a γ-globulin of at least 1.8 g./100 ml. were studied separately The mean γ globulin value was ? 15 g./100 ml. The mean age of the patients was 19.5 years (range 9 to 47 years) There were 7 males and 9 females

Three of the patients had jaundice one had thrombopenic haemorrhages, one had exanthema, one had peritonsillar abscess, and one had a very prolonged disease with lung infiltration

In 13 of the cases electrophoretic examination was repeated. In all of these cases the γ fraction had regressed—in the two cases with the highest γ value 3 6 and 3.5 g./100 ml. to 1 0 and 1 4 g./100 ml. respectively In none of the cases were any persisting sequelae from the disease observed.

COMMENTS

A large group of definite P B positive mononucleosis and a smaller group of P B negative cases of the disease were studied. Although of the latter group patients above 20 years and without evident tonsillar changes may represent some other disease the pro-

tein changes as well as the cytological changes were essentially the same as in typical infectious mononucleosis.

The clinical picture in all these cases of mononucleosis with and without a positive P B reaction was similar and characterized by the following phenomena

1) A primary inflammatory reaction which despite relatively long fever was weak, as judged from the number of polymorphonuclear cells, the haptoglobin and fibrinogen levels. It was followed a few weeks later in about half of the cases by abnormally low values for these three factors.
2) A secondary reaction with an increase of the γ fraction, and of the P B titer both often persisting for a long time. Other manifestations of this reaction were the almost regular enlargement of the spleen and of the lymph nodes, and finally the increase of the number of mononuclear cells in the blood with the appearance of atypical cells. These changes form the characteristic clinical picture of the disease.
3) The third component of the picture in mononucleosis consisted of signs of a slight hepatocellular damage with regularly increased serum aldolase and, in a small percentage of cases, jaundice. There was also increased serum alkaline phosphatase and pathological bromsulphalein test, changes which were only sporadically checked in the present material.

Comparison of the cases of mononucleosis with those of hepatitis shows that the former with their intense throat affection were initially accompanied by a relatively stronger primary inflammatory reaction. But in the later course (Fig. 19) they distinctly more often showed a reduction of the number of polymorphonuclear cells, of haptoglobin and fibrinogen than the cases of hepatitis. The early marked increase in the γ-fraction and the thymol turbidity persisting for a long time, were common to both diseases, and also the milder and shorter course in children than in older patients. But the *picture of mononucleosis was characteri ed above all by the lymphoid proliferation and tonsillar changes that of hepatitis mainly by the hepatocellular damage*

Prognosis All patients studied recovered. The 16 patients with a large γ fraction, at least 1.8 g./100 ml., had a somewhat higher mean age and a more severe acute picture with more complications than the other patients, but no sequelae were noted. This is in agreement with the findings of BENNIKE (1960) for example, who found that the mononucleosis was not an initial step in the formation of cirrhosis. The slight destruction of liver parenchyma in mononucleosis is evidently not sufficient to initiate the cirrhotic process.

VIRUS ENCEPHALO-MENINGITIS

As mentioned in Chapter I, in poliomyelitis some investigators have found an increased, others a normal γ fraction. In tracheotomized patients with

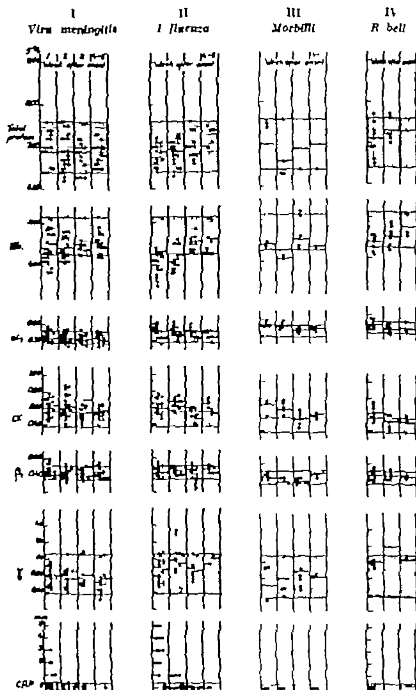


Fig 23 Various virus fect ons.

Diagram I Plasma prot in changes in viru enc phalo-meninelt
● 27 cases of iru meningit of generall nsettled origin.
○ 10 cases of probably polium 1 t

severe poliomyelitis JUNGNER & JUNGNER (1960) found a moderate decrease of the albumin and an increase of the a_2 fraction and of the fibrinogen values but only a slight increase of the γ-fraction.

In virus meningitis other than poliomyelitis the serum protein changes are only slight and consist mainly of a small increase in the a_2 fraction and slightly decreased serum albumin (WIDELL 1958).

Material. The material consisted of 62 cases of lymphocytic meningitis with a favourable course. Eleven of the patients had mumps meningitis, 2 had ECHO infection, 7 had R. S. S. E., *i.e.* tick borne encephalo-meningitis, and 10 paretic cases were diagnosed as poliomyelitis although no attempts were made to isolate the virus—2 of these patients were tracheotomized. Twenty five cases were lymphocytic meningitis of unknown origin. The mean age of the patients was about 20 in all of the groups except the R. S. S. E. group with a mean age of 41. Most of the patients in this group and in the polio group were men.

Findings. Nearly all the patients had fever on the average for one week, but the patients with R.S.S.E., usually for 2 weeks. The latter group as well as the patients with polio often showed a slight increase in the number of polymorphonuclear cells in the blood, but in other cases generally no blood cell changes were found. The E. S. R. did not usually exceed 20 mm., the α frac

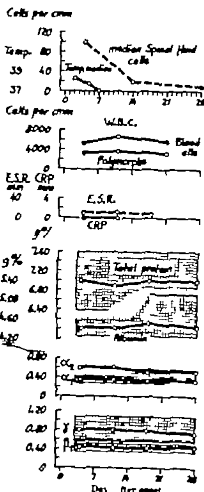


Fig. 21. Mean curves for changes in 27 cases of virus encephalo-meningitis of generally unsettled origin. Chequered zones as in Fig. 4.

Diagram II. Plasma protein changes in *influenza*.
● 16 cases of type A Singapore influenza.
○ 8 cases of type B influenza.
Diagram III. Plasma protein changes in 14 cases of *morbilli*.
Diagram IV. Plasma protein changes in 13 cases of *rubella*.
Chequered zones as in Fig. 3.

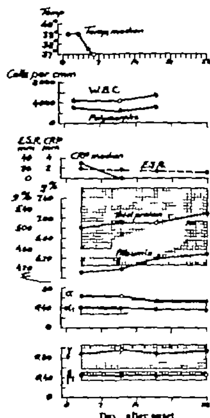


Fig. 25. Mean curves for changes in 24 cases of *infl e a*.
Chequered zones a in Fig. 4

tions were only slightly increased and the CRP was usually absent in the serum also during high fever Some cases of mumps meningitis with associated orchitis had CRP in the serum and considerable changes in the *α fractions* and *elevation* of E. S R. The primary inflammatory reaction as reflected by α_1-increase and E. S R. elevation was also moderately strong in R. S S L and prolonged in some of the cases of polio

The scatter diagram (Fig. 23) is based on the cases of polio and cases of virus meningitis most of them of unknown aetiology The mean curves (Fig 24) are based only on these cases of virus meningitis, which were ?7 in number

The figures show that the fever and spinal fluid pleocytosis were accompanied by an insignificant change in the α and slight increase in the α_1 fraction and the E. S R In addition, no regular change was found in the albumin the β-fractions and the γ fraction in these cases Also in the cases of mumps meningo-orchitis, poliomyelitis and R. S S E. the γ fraction did not increase in spite of their somewhat stronger and more protracted primary inflammatory reaction but a significant rise of the antibody titer was noted in many of them

Comments The active process in virus meningitis is accompanied by only a weak primary inflammatory reaction, apart from the frequently high fever and by no evident signs of a secondary reaction, apart from the formation of specific antibodies No obvious lymphoid proliferation or increase of the γ fraction was noted.

INFLUENZA

WUHRMANN & WUNDERLY (1957) reported slight plasma protein changes in influenza, like FERRUCCI (1957) in *influenza A Singapore.*

Material The material consisted of 10 cases of influenza A Singapore and 8 cases of influenza type B In about half of the cases the diagnosis was made serologically The mean age of the patients was 38 years (range 10—70 years) No cases with complicating

pneumonia or purulent sinuitis were included

Findings All the patients had fever for 3—8 days. In all except 3 the E. S R. was slightly elevated. The CRP was demonstrated in all cases of type A but only in a few of type B In some cases the polymorphonuclear cells in the blood showed a slight increase in number but in about half of the cases the number was below the normal *mean, 3 000 cells/cu. mm.*

The scatter diagrams (Fig 23) show that the changes in cases of type B were smaller than in cases of type A, but the differences were insignificant, so that both groups were taken together as a basis for the mean curves (Fig. 25)

These curves show a moderate increase of the α fractions, and a slight decrease of the albumin. The γ fraction remained, on the average, unchanged throughout the course of the disease though a slight increase was found in some cases.

Comments The influenza virus is injurious to the tissues invaded—the *mucosa of the respiratory tract.* After a short incubation time a moderate primary inflammatory reaction followed but except for the formation of specific antibodies, as a rule, only slight signs of a secondary reaction appeared

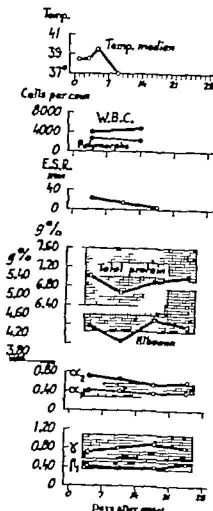


Fig. 26. Mean curves for changes in 14 cases of *mo billi.*
Chequered zones as in Fig 4.

MORBILLI

According to WUHRMANN & WUNDERLY (195) uncomplicated morbilli is accompanied by only slight inflammatory alterations of the serum proteins. NICOLA (1956) and EWERBECK (195) on the other hand, have seen an increase of the γ fraction in the after course of the disease

Material The material consisted of 14 patients, aged 2—34 years, median age 10 years some of them were seen only in the later course. The diagnosis was firm in all. Cases complicated with

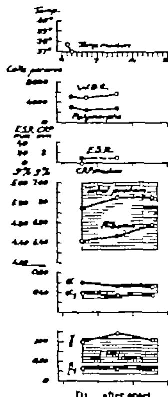


Fig 2? Mean curves for changes in 13 cases of *rubella*
Chequered zones as in Fig 4.

pneumonia or purulent infection of the airways were not included. Most of the patients received antibiotic therapy

Findings All the cases ran a very similar course The fever preceded the rash by some days and lasted, on the average 8 days. The findings are given in the scatter diagram (Fig 23) and in mean curves Fig 20)

CRP was found in the serum of the adults, but usually not in that of the children. The values for W B C. and for polymorphonuclear cells were on the average below the normal mean during the whole course—also the number of mononuclear cells was somewhat low the first week.

The E. S R. and the α fractions were moderately increased during the first 2 weeks. The albumin was decreased during the 2nd week and the β fraction was often slightly decreased during the 2nd—3rd week, an unusual finding in other diseases in this material. The γ level was, on the average not elevated even in the aftercourse—only 2 adults showed a slight increase.

On the other hand, in cases which were complicated by supervening bacterial infection, the number of polymorphonuclear cells and the α fractions were markedly increased, and the albumin decreased. But even these cases were generally without any increase of the γ fraction.

Three cases of *varicella* studied had a moderate increase of the α fractions, but the γ fraction was normal.

RUBELLA

Only few data are available on the serum protein in German measles. According to WUHRMANN & WUNDERLY (195) the changes are only slight and consist mainly of a small increase of the α fractions and sometimes a positive thymol turbidity test. The increased number of plasma cells in the blood and of plasmoblasts in the lymph nodes, which MOESCHLIN (1940) found prompted him to search for changes in the blood proteins in this disease but without success by means then available.

Material The material consisted of 13 cases of German measles in patients, aged 14—30 years, with a me-

dian age of 20 years 10 were females. As many as half of the patients had joint symptoms which is a relatively high frequency for this complication, possibly because of the relatively high number of adult females in the material. Six of the 7 with joint symptoms were females above 18 years.

Findings The findings are given graphically in the scatter diagram (Fig 3) and in mean curves (Fig 27) The slight elevation of temperature was accompanied by a slight or no change of the α-fractions, the albumin or the E. S. R. and usually by a slight decrease in the number of polymorphonuclear cells. In none of the cases examined was the CRP demonstrable. Despite the weak primary inflammatory reaction the γ-fraction was slightly increased in 6 of the cases, with a maximum already in the second week most of these patients were adult women with joint symptoms.

Comments In contrast to morbilli, several of these cases of rubella showed an increased γ fraction. This may be in accord with more distinct findings of lymphoid proliferation in rubella as manifested by enlargement of lymph nodes and the appearance of plasma cells in the blood However this series may be a selection of somewhat more severe cases. The real frequency of γ-elevation in rubella may be less than this series suggests.

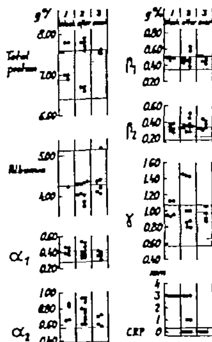
STOMATITIS APHTOSA

Stomatitis aphtosa is the usual primary manifestation of infection with Herpes Simplex virus. In this disease STANKNEC et al (1959) found plasma protein changes of the acute inflammatory type according to WUHRMANN & WUNDERLY

Material The material consisted of 10 cases of stomatitis aphtosa, some of which were unusually severe In only one case was an attempt made to confirm the diagnosis serologically In that case the titer of complement binding antibodies against Herpes simplex was definitely increased. The patients were on the average, 20 years of age (range 11—39 years) The disease was characterized by stomatitis and fever on the average, for 10 days. Two patients also had conjunctivitis and one myocarditis with definite E. C. G. changes.

Findings All the patients had fever and all were found to have CRP in the serum. In most of them the W B C. was moderately increased owing to an increase in the number of polymorphonuclear cells, and they had a distinct and protracted increase of the α-fractions and a decrease of the albumin (Fig 28) A slight to moderate increase of the γ fraction occurred in half of the cases already in the first 2 weeks of the disease. Most of the patients had an increase of the β fraction, some also of the β fraction

Comments. These cases of aphtous stomatitis were associated with a marked primary inflammatory reaction, but many of them also with a secondary reaction in the form of a γ-elevation and still more often a β elevation. The total protein was somewhat high in many of the cases. Haemoconcentration because of difficulties in drinking may be partly re-



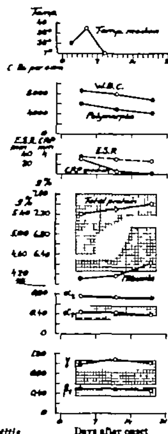


Fig. 28. *Aphtous stomatitis.*

Fig. 28 a. Plasma protein changes in 10 cases.
Chequered zones as in Fig 3.

Fig. 28 b. Mean curves for changes in 10 cases.
Chequered zones as in Fig. 4.

sponsible. The present small series, like most of this virus infection series, is a selection of individuals, older and probably more severely ill than the true mean in the general population. This is illustrated by the case in a woman, 20 years of age with a myocarditis an unusual complication of aphtous stomatitis.

PRIMARY ATYPICAL PNEUMONIA AND ORNITHOSIS

According to WUHRMANN & WUNDERLY (195) the plasma protein changes in atypical pneumonia are the same as in bacterial pneumonia. They believe that the cold agglutination, which is demonstrable in about half of the cases, is closely connected with an increased γ fraction.

It has been found that pneumonia associated with cold agglutinins in the serum is caused by very small, pleuropneumonia like organisms, EATON agents (MUFSON et al. 1961) which seem to be a kind of mycoplasma (FREUNDT 1958) and not a virus Ornithosis, on the other hand is caused by

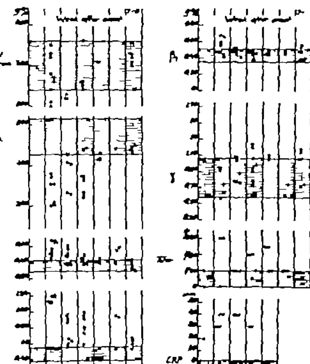


Fig. 29 Plasma protein changes in *primary atypical pneumonia and ornithosis.*
Chequered zones In Fig. 3.
● 14 cases with cold agglutinins in the serum.
O 3 cases with elevated C.F. titer for lymphogranuloma venereum.
Titer scale- ● cold agglutinins.
O C.F. titer for ly.gran. vener. antigen.

large viruses related to the rickettsiae (MEYER 1950). The two agents display a similar sensitivity to certain antibiotics.

Material. The material consisted of 14 patients with a positive cold agglutination test and roentgenologic signs of pneumonia. Most of the patients had a troublesome cough, the fever did not respond to penicillin, and was usually of long duration, on the average 20 days. The average age was 43 years (range 11—73 years). In addition, the material included 3 cases of probably ornithosis with roentgenologic signs of pneumonia and with complement fixing antibodies in the serum against lymphogranuloma venereum antigen, more or less definitely suggesting an active ornithosis. The 3 patients had all been exposed to diseased birds, and their pneumonia was resistant to penicillin.

Findings. All of the patients had CRP in the serum and in all the E.S.R. was substantially elevated. The scatter diagrams and mean curves (Figs. 29 and 30) show the findings made in these cases. The changes in the 3 cases of ornithosis coincided largely with those of the cases with cold agglutinins. The mean curves are based on all 17 cases of pneumonia.

The patients had a marked primary inflammatory reaction with a large increase of the α fractions, marked elevation of the E. S. R., a large amount of CRP and a decrease of the albumin, all disorders equally severe as in bacterial pneumonia. The num

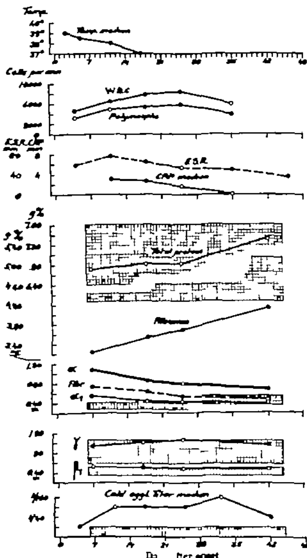


Fig 30 Mean curves for hanges in 17 ca es f primary atypical pneum ni (and ornith sis)
Chequered ones as I Fig 4.

ber of polymorphonuclear cells, on the other hand, increased only slight ly and then in the later course of the disease. About half of the patients had a slight to moderate increase of their γ fraction. The mean curves for fraction and for cold agglutinin titer were flat without any distinct maxi mum.

Nearly all cases in which fresh sera were examined and many in which *only frozen sera were used showed an* increase also of the β fraction. The β fraction, in some of the cases, was distinctly increased

Comments The cases of primary atypical pneumonia and ornithosis displayed in their clinical picture a pri

mary inflammatory reaction as strong as in bacterial infection but no distinct leucocytosis. As in severe cases of bacterial disease, in atypical pneumonia the γ fraction was often, and the β_1 fraction most often, increased but only very moderately

TOXOPLASMOSIS

Lymphadenitis (SIIM 1961) often starting relatively abruptly with slight fever but rather persistent, is the main *clinical manifestation of a toxoplasmosis* acquired after the newborn period.

Toxoplasmosis is associated with a lymphocytic blood picture, often with a small number of atypical round cells resembling McKinlay cells and sometimes with enlargement of the spleen the thymol reaction is often positive. The blood protein pattern often shows an isolated increase of the γ globulin (BELFRAGE & BERGDAHL 1957)

Material The material consisted of 25 patients, aged 5—60 years (average 28 years) Two thirds of the material were females. The diagnosis was based on a Dye test titer of at least 1/1250 a corresponding rise in complement fixation titer and a variation of the titers with the clinical course. At least 2 serological tests were performed for each individual. In 2 cases it was possible to isolate toxoplasma from lymph node tissue (Dr SIIM, Copenhagen)

As many as 3 cases with a definite increase of the titer for toxoplasmosis *with fever and enlarged lymph nodes* also showed a definite increase of the titer for Paul Bunnell test after absorption, and a further case showed an increase of the titer against Tularense

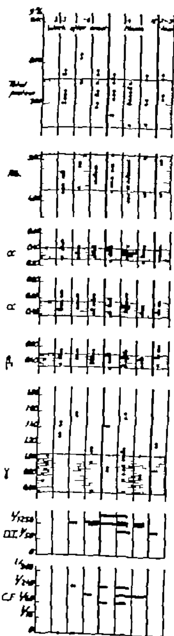


Fig. 31 *Toxoplasmosis.*
Plasma protein changes in 21 cases mainly of lymphonodular type.
Chequered zones as in Fig. 3

antigen as well as a histologic picture arguing for tularaemia in the extirpated lymph node from which, however Dr SIIM succeeded in isolating toxoplasma These 4 cases, in which double infection is possible were not included in the series.

The remaining 21 cases were seen for the first time somewhat late in the course usually several weeks, sometimes 2—3 months after onset The clinical picture was that of lymphonodular toxoplasmosis in all, except one or two cases with a typhoid like picture. The disease was mild in most cases Many of them were followed up at the out patient department during the entire course All recovered some after more than one year of persistently swollen lymph nodes and tiredness.

Findings. The scatter diagrams and the mean curves (Figs. 31 and 32) show an infection with slight or no primary inflammatory reaction. As mentioned, the patients were, however as a rule not examined during the first 2 weeks of disease No increase occurred in the number of polymorphonuclear cells, and the a fractions and fibrinogen were unchanged or only slightly increased. On the other hand, a relative but as a rule not absolute increase occurred in the number of mononuclear cells in the blood and in two thirds of the cases also an increase of the fraction, which was often the only plasma protein change for a long period. Many cases also had a slightly elevated thymol turbidity value The γ maximum occurred about 4—6 weeks after onset and then the - fraction slowly decreased The Dye test titer and especially the complement fixation titer increased somewhat later than the γ fraction and reached their maxima in the third month

The total protein was usually somewhat high during the first two months as in the after-course of hepatitis and mononucleosis.

Comments. The picture of this disease caused by a parasite was thus similar to that of hepatitis and mononucleosis with signs of a lymphoid proliferation and with a γ increase as the most striking plasma protein change in other words, a slight primary inflammatory reaction but a marked secondary reaction.

MYOCARDIAL INFARCTION

A short section on myocardial infarction is added to this report on infections as an example of a condition caused by abrupt ischaemic necrosis.

As mentioned in Chapter I myocardial infarction gives rise to a primary inflammatory reaction with corresponding changes of the protein pattern and with fever and leucocytosis (FORSSMAN 1954) The γ and β-fraction are described as usually normal by LINKO and co-workers (1950) while WITAS (1953) found the latter slightly increased.

Material The material on which the scatter diagram and the mean curves are based, consisted of 15 cases Data *on further 9 cases were collected for* certain statistical analysis (Fig. 49 50 and Table 20)

All of the cases were diagnosed clinically with electrocardiography which

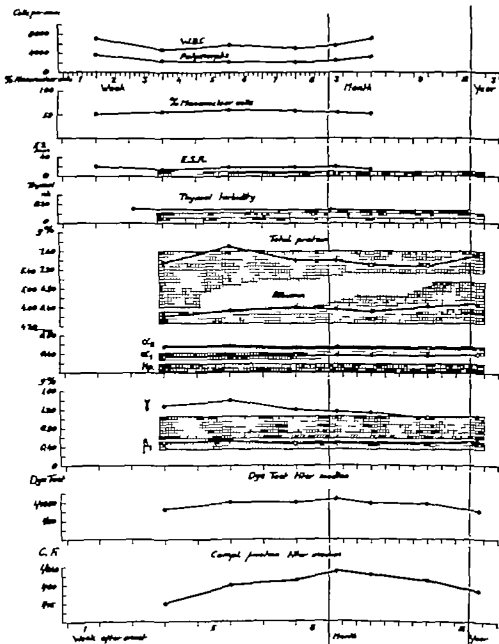


Fig 32. Mea curves for the ha ges in 21 cases of tox plasmosis. Chequered zones as in Fig. 4.

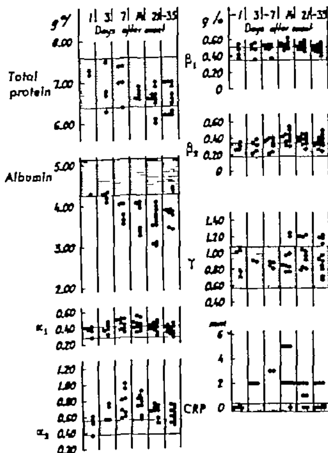


Fig. 33. *Myocardial infarction.* Plasma protein changes in 15 cases. Chequered zones as in Fig. 3. All sera had been kept in the frozen state for several weeks before examination.

showed a pathological Q wave in all except one mild case and S-T changes of typical appearance and course. Two of the cases were very mild and practically afebrile. In the remainder the body temperature was distinctly elevated. CRP was regularly found in the serum. All survived the acute phase. The mean age of all the 24 patients was 65 years (range 50—80). There were 1[illegible] males and [illegible] females.

Some of these elderly patients probably also had some other disease particularly hypostatic pneumonia, capable of influencing the protein pattern.

Findings. The results are apparent from the scatter diagram (Fig. 33) and the mean curves (Fig. 34). The mean curves show distinct leucocytosis already on the first day but no obvious fever and no CRP in the serum until the day after infarction. The α fractions and E. S. R. increased rapidly the first few days to reach their maxima the 8th—10th day after the infarction. A marked increase of the α_2-fraction persisted for a long time but the increase of the α_1 fraction was usually slight. The albumin showed a corresponding fall which was conspicuous and resulted in a slight

decrease of the total protein. The β_1 fraction increased with a maximum in the 2nd week. This increase was evident although the sera studied had been kept in the frozen state. The mean for the γ fraction in this material of elderly patients was slightly above the mean for young normals from the beginning and showed an insignificant increase in the 3rd week. The spread of the γ values was wide. During the course the γ fraction decreased in 9 and increased in 5 of the cases represented in the mean curves.

Comments. The mean curves for the *temperature resembled those in* FORSSMAN's large series of myocardial infarction, which suggests a similar average severity. In both series fever appeared about one day but leucocytosis already some hours after infarction. In bacterial disease such as pneumonia and also after injection of endotoxin both signs appear simultaneously. It is possible that some products from the region of infarction which are not primarily pyrogenic are involved in the causation of leucocytosis as well as of the blood sugar increase after infarction.

The myocardial infarction, *i.e.* an ischaemic necrosis does not imply any action of exogenous antigen. The infarction was followed by a moderate primary inflammatory reaction but not by any regular secondary reaction in the form of an increase of the γ fraction, a β increase however seems to have been an almost regular consequence.

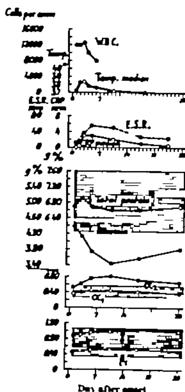


Fig. 34. Mean curves for changes in 15 cases of *myocardial infarction.* Chequered zones as in Fig. 4.

PLASMA PROTEIN CHANGES AFTER INTRAVENOUS INJECTION OF ENDOTOXIN

Fever can be induced in man and in animals by injection of endotoxin into the blood stream It can be given as a constituent of an emulsion of Gram negative bacteria or as pure lipopoly saccharide (for survey see EICHENBERGER et al 1955) Endotoxin acts upon the leucocytes, which release an endogenous pyrogen This appears to be the factor directly responsible for stimulation of the temperature centre of the brain (WOOD JR. 1958 HERION et al. 1961) However bacterial pyrogens introduced intrathecally seem to provoke fever also by a direct action on the brain (BENNETT JR. et al. 1957)

The phenomena accompanying endotoxin fever are as follows. An initial decrease of the polymorphonuclear cells occurs during the first few hours after the injection of a large dose of pyrogen. This is followed by an abrupt rise of the temperature with an increase in the number of polymorphonuclear cells, while the mononuclear cells show a corresponding slight decrease

During the first hours after the injection fibrinolysis occurs (EICHENBERGER et al 1955 NILSSON et al. 1961) In the further course the fibrinogen as well as the α fractions increase (HEDLUND et al. 1948) A decrease of the serum iron, a relatively less marked decrease of the transferrin and a somewhat later increase of the serum copper have also been described (BRENDSTRUP 1953)

In addition the early appearance of CRP in the serum has been noted (HEDLUND et al 1948) Agglutinating antibodies have been demonstrated in rabbits in the aftercourse but the γ value in humans does not increase after administration of pyrexal® which consists of pure lipopolysaccharide from Salmonella abortus equi and is also active as O antigen

AUTHOR'S EXPERIMENTS

A small series of experiments was carried out with induced fever in human volunteers. The purpose was to ascertain the time table and the interrelationship between the same disorders as those studied in the acute infections, but usually not on the first few days of the disease The purpose of the experiments was thus to elucidate the first stage of an active injurious process with the assumption that this stage is in principle similar

whether it is caused by intravenous pyrogen or a bacterial infection

Material The material consisted of 6 young healthy males and one young man with a smouldering staphylococcus aureus infection, who was given endotoxin twice in the course of 2 months.

Method In 6 experiments the subjects received an injection of Pyrifer® an emulsion of killed E. coli and in 2 Pyrexal® as mentioned pure lipopolysaccharide.

The white blood cell picture was followed by determining the W B C and differential count, and the blood protein pattern by electrophoresis, determination of the total protein, haematocrit fibrinogen, haptoglobin, CRP protein bound hexoses and coeruloplasmin. In one case the serum Cu was also followed. During the first day samples were collected for study every third hour during the following 3—10 days at longer intervals.

In Fig 35 all changes are given as deviations from the original value. The temperature, blood cells and CRP are given in absolute values other plasma protein data are given as values relative to original values after they had first been calculated as percentages of the simultaneously noted total protein —this to eliminate the effect of changes in the extracellular fluid during the experiments.

Two of the volunteers were given I^{131} albumin—15 μ C intravenously—10 and 15 days, respectively before the experiment. The radio-activity in the serum and urine was assessed every day until some days after the experiment During this time the subjects received iodide daily by mouth

In 6 of the experiments, studies on the fibrinolytic activity of the plasma were performed at the same time as fibrinogen determinations by Dr I M Nilsson

FINDINGS

The findings in 3 experiments are given in Fig 35. All of the injections of pyrogen resulted in *fever* In one case the temperature rose to only 37.8 C and in the others to 38.5°C—39.5 C. Fever began with a chill 2—5 hours after the injection. In most of the experiments with Pyrifer the temperature curve was biphasic. In experiments with Pyrexal the onset of the fever was more abrupt but the fever was of shorter duration than in the Pyrifer group

In all the patients studied the elevation of the body temperature was associated with an increase of the number of *polymorphonuclear cells* an initial neutropenic phase was noted in 2 cases. During the first 24 hours the mononuclear cells showed a slight decrease which was strongest at the time when the fever reached its peak.

In all these cases *CRP* appeared in the serum in relatively large amounts. CRP began to appear already 5—10 hours after the injection and reached a maximum after 24 hours, to disappear within 4—7 days.

The *α fractions* showed a distinct increase only in the 2 volunteers who had most marked pyrexia The α_1 fraction did not increase at all in the volunteer in whom the fever was very

Fig. 33 *Experiments with endot xin in 3 individuals.*
Curves for various changes after injection of pyrifer®. Protein changes, other than CRP calculated as part of the t tal protein and expressed relati to the original value before injection the absolute values f other changes expressed in the same way

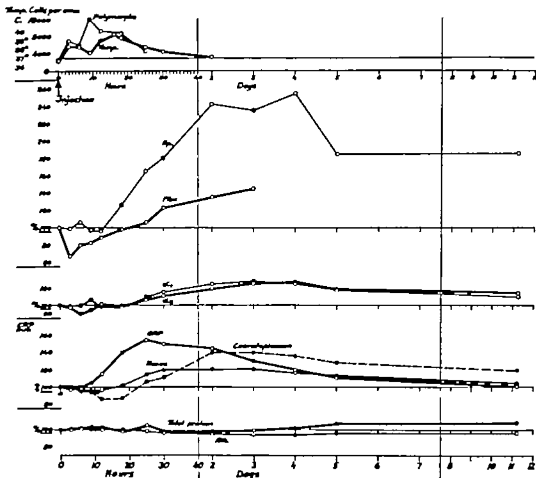


Fig 33 Experiment in a healthy indi idual.

slight the α_1 fraction did not increase in those cases in which the haptoglobin increased only insignificantly

The increase of the α_1 fraction began most often already within 12 hours, the α_2 fraction somewhat later Both reached a maximum 2—4 days after the injection The α_1 fraction became normal, as a rule 7 days after the injection, while α_2 never became normal during the observation period of 4—11 days. The α_2 increase was preceded in most cases by a slight relative decrease during the first 12 hours.

The *haptoglobin*, like the α_2, was unchanged or showed a slight decrease during the first hours after the injection—the absolute haptoglobin values showed a slight decrease in half of the

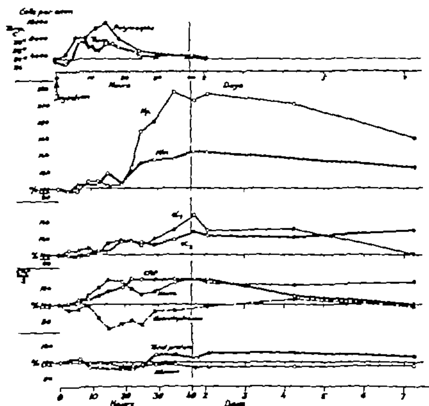


Fig. 35 b Experiment in healthy individual.

cases. Then an increase occurred during the second 12 hours in all cases, with a maximum 2—3 days after the injection. In most cases the elevation was still demonstrable at the end of the observation time on the 7th—14th day

Only a slight absolute increase of the haptoglobin was found in cases with a low original haptoglobin and in the 2 experiments with the chronically infected person.

The *fibrinogen* varied largely in the same way as the haptoglobin. In most cases there was an initial decrease during the first few hours before and during the steep rise of the temperature—as a rule, also the absolute values decreased. Then an increase followed with a maximum 2—3 days after the injection. It sometimes disappeared before the 4th day in other cases it was still persistent at the end of the observation period, *i.e.* 4—7 days.

The chronically infected person showed only a slight increase of the fibrinogen and of the haptoglobin for the degree of fever

Protein-bound hexoses increased in all the cases studied, also markedly in both experiments with the chronically

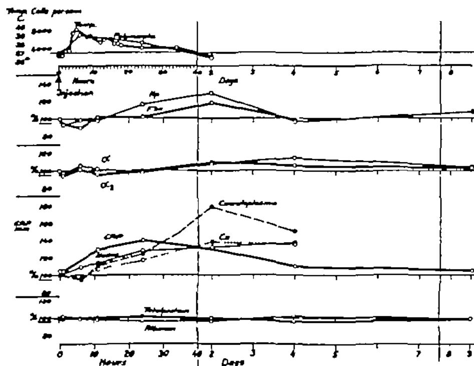


Fig. 35 c. Experiment in chronically infected individual.

infected patient. The increase started in all but one case within 12 hours, it culminated 1—4 days after the injection, and, in all but one case, regressed within 4—11 days after the injection.

The *coeruloplasmin* showed an initial decrease—also of the absolute values, a decrease that lasted somewhat longer than that of the fibrinogen, and in some of the experiments it was largest during the actual peak of the fever.

As a rule, it was followed by an increase which was largest 3—4 days after the injection and was usually still demonstrable at the end of the observation period, i.e. the 4th—11th day. Patients with a slight initial decrease showed a relatively early and relatively marked increase and *vice versa*.

Finally the serum *copper* was followed in one of the experiments with the chronically infected patient. The level of this metal varied with the coeruloplasmin with a slight initial decrease and then a prolonged increase.

The *albumin* was slightly decreased from the day after fever induction with a minimum on the 2nd—3rd day. It had not recovered original values by the end of the observation period on the 7th—11th day. The chronically infected case showed hardly any change in the albumin.

The *total protein* increased during the first hours in half of the cases,

usually very slightly but markedly in that individual who only had a slight increase of temperature and who was ambulant the whole time

During the second 12 hour period the total protein decreased and was lowest after 12—36 hours, but no decrease was noted in the chronically infected or the above mentioned person without distinct fever

Of the other protein fractions in cases with high fever the *β fraction* showed a tendency to increase after 2—4 days, while the *γ* fraction showed no definite change

Of the 2 patients who received a small dose of I^{131} *albumin* before the experiment the body temperature rose markedly in one (max. 39 C) and slightly (max. 37 7 C) in the other

During the febrile reaction in the patient with high grade fever the serum radio-activity was found to decrease distinctly below the expected value Thi decrease paralleled the changes of the serum albumin, total protein and haematocrit with a minimum 12—14 hours after the injection and a return to normal after 4—5 days. However the values for the radio-activity did not reach expected levels in the following examinations, not even when allowance was made for the 6— % loss of the total protein in association with blood sampling in the course of the experiment. During the day of fever the radio-activity in the urine was significantly higher than the expected value These findings suggest that a dilution of the blood during the days after the injection of pyrogen was mainly responsible for the decrease noted in the serum activity but that the degradation of albumin during the day of fever was also increased as shown by the increase in the activity of the urine on that day

The person, in whom only slight fever was induced, showed no decrease but a distinct increase of the radio-activity above that calculated, concurrently with a simultaneous transient increase of the serum albumin and total protein and haematocrit, all with a maximum 7 hours after the injection and all with return of the activity to expected level after 1—2 days. Neither did the radio-activity in the urine increase during the day of the experiment Thus, there was only evidence of haemoconcentration ascribable to the fact that the person in this experiment was ambulant throughout the experiment.

The *fibrinolytic activity* showed a definite increase in all experiments in which it was studied. It started somewhat earlier than the rise in body temperature and, as a rule reached its maximum at the same time as the latter but then soon regressed (Dr I M Nilsson)

COMMENTS

The purpose of these experiments was to illustrate the time table of the different disorders and their interrelation in the first stage of an acute injurious process. similar to an infectious disease

In a *micro-"model"* of an acute infection by staphylococcus aureus of chorio-allantois membrane from chicken's eggs Jacherts (1960) has

PART V

FURTHER REMARKS ON CHANGES IN ACUTE INFECTIOUS DISEASES

In acute infection changes were as a rule observed in the body temperature in the blood cell picture and in the plasma protein pattern. The severity of these changes in different types of acute infection are accounted for below while their interrelationships will be presented in Chapter IV

BODY TEMPERATURE

The body temperature was elevated except in many cases of hepatitis, rubella and toxoplasmosis. The fever was severe in most of the cases of acute infection in this hospital series Low grade fever was common in hepatitis. It was also noted in some cases of mononucleosis, biliary tract infection and enteritis.

BLOOD CELL PICTURE

Bacterial infections were most frequently accompanied by leucocytosis due to an increase in the number of polymorphonuclear cells, which persisted until the fever ceased Exceptions to this are given in Table 10 The number of white blood cells in the presence of fever was usually normal or decreased in salmonellosis especially of the typhoid type and in extrapulmonary tuberculosis.

In most viral infections no increase in the number of polymorphonuclear cells was observed. The exceptions to this rule are given in Table 11 Slight leucocytosis was often noted in cases of R. S S E. and poliomyelitis.

The number of mononuclear cells in the blood in the febrile stage of acute bacterial infection was, as a rule normal or slightly decreased while relative lymphocytosis was often noted in the later course In virus infections relative lymphocytosis was often recorded already in the initial febrile stage because of the simultaneous polymorphopenia. The picture observed in mononucleosis was unique with its marked, absolute increase and qualitative change of the mononuclear cells. A slightly raised number of "large lymphocytes was, however also seen in many other conditions.

PLASMA PROTEIN PICTURE

The changes in the plasma protein picture noted in acute infections were in principle of four types namely increase of the *α fractions fibrinogen and CRP* increase of the *γ fraction* increase of the *β-fractions* and a decrease of the *albumin*.

Table 10 *Absence of leucocytosis in the acute stage of bacterial infection*
(W.B.C.≤6,000 per cu mm during fever≥38.0)

	Number of cases without leucocytosis	Total material
Salmonellosis	10 (especially typhoid and paratyphoid)	19
Tuberculosis	6 (extrapulmonary tuberculosis)	12
Biliary disease	6	22
Septicaemia	2 (subacute bacterial endocarditis)	7
Bacterial pneumonia	6	57
(Primary atypical pneumonia	7	14)

The increases of the *a fractions fibrinogen and CRP* were components of the primary inflammatory reaction. These changes were marked in bacterial infections. Table 12 shows that the *a* proteins were particularly increased in cases of pneumonia and septicaemia and the a_2 fraction also in diseases secondary to streptococcal infection, such as rheumatic fever and some cases of erythema nodosum. These disorders were generally slight in virus infections. A reversal of the a_1/a_2 quotient occurred in some cases of hepatitis and mononucleosis.

In bacterial infections the *γ-globulin* was increased only in severe and protracted cases, and in secondary disease after streptococcal infection. In the virus infections the γ fraction was usually normal except in hepatitis and mononucleosis. Rubella was likewise often accompanied by an increase in the γ fraction. This also applies to the parasitic disease toxoplasmosis.

The increase in the γ fraction was marked in cases of hepatitis and mononucleosis, but also in several cases of bacterial pneumonia, biliary disease and acute rheumatic disease (Table 12).

A γ fraction below normal was also sometimes noted in different types of bacterial and virus infections, and then, as a rule, in mild cases in young persons.

The β-fractions changed less regularly than the other fractions in acute infection. The change nearly always consisted of an increase.

A marked increase of the *β fraction* (Table 12) occurred in diseases with involvement of the liver such as hepatitis, mononucleosis and gall stone. An increase was noted in these diseases also in many cases with the

Table 11 *Polymorphonuclear leucocytosis in the acute stage of virus infection*
(polymorphs > 8,000 per cu mm)

	Number of cases with leucocytosis	Total material
Viral meningitis	14 (especially R.S.S.E. and poliomyelitis)	56
Mononucleosis	9 (1st week)	91
Morbilli and Influenza	7	49
Stomatitis aphtosa	7	10

Table 12. *Frequency of cases with markedly abnormal protein fracti ns (g/100 ml) in various diseases studied*

	Number of cases exam.	Total protei ≥8.5	γ ≥2.00	$β_1$ ≥0.65	$β_2$ ≥0.5	$β_2$ normal γ≥1.00	γ≥	Albumin ≤2.50	$α_1$ ≥0.70	$α_2$ ≥1.20	γ ≤0.53	Total protei ≤5.5
Disease secondary t treptococcal infection	39	1	5	3	3	3			1	8		
Chron. bacterial infection	70			1	1	1	1		1	1		
Bacterial pneumonia, pleuritis	74	2	6	2	6	1		10	10	6	2	1
Tonsillitis	40			1		1						
Septicaemia, abscessus	10		1		1			4	2	2	1	2
Bacterial meningitis	12		1		1			4	1	1		4
Enteritis, colitis	50					1		3			4	3
Urinary tract infection	11				1				1	1	1	1
Infectious r toxic skin disease	40								1	2	5	4
Resp. tract infection	14										5	3
Viral enc. meningitis	63	1		1							5	1
Influenza, atypical pneumonia	48									1		
Exanthematic iral infection	51										1	
Biliary disease due t gall stone	83	2	6	4	11			4				
Infectious hepatitis, epidemic cases	51	5	6	5		1	3	1				
T ic hepatitis	6			3								
Other cases of hepatitis	6	5	12	4		3	2					
Infectious mononucleosis without jaundice	134	8	5	4	1	4	4					
Infectious mononucleosis with jaundice	13	1	1	3		1	1					
Toxoplasmosis	25			1	1	1	1					
	879	25	45	32	26	17	12	26	17	22	1	19

Table 13 *Increase of beta globulin/s in various infections with and without hypergammaglobulinaemia*

(All values derive from examination of fresh sera)

	Cases with normal gamma			Cases with increased gamma > 1.10 g%		
	number of cases			number of cases		
	studied	thereof with		studied	thereof with	
		β_1 increase > 0.31 g%	β_2 increase > 0.34 g%		β_1 increase > 0.31 g%	β_2 increase > 0.31 g%
Bacterial pneumonia	16	3	7	16	10	16
Tonsillitis	12	5	8	13	6	9
Biliary disease	30	14	23	37	19	31
Hepatitis	6	4	0	49	33	35
Mononucleosis	11	5	3	48	27	31
Viral meningitis	42	6	4	4	2	3

γ fraction normal (Table 13) In a few cases of disease secondary to streptococcal infection the β fraction was also markedly increased.

The β fraction was augmented in most cases in which the γ fraction was increased and also in many cases with the γ fraction normal, particularly in cases of gall stone and various kinds of bacterial disease (Table 13) In these diseases the β_2 fraction was often markedly augmented (Table 12) In some cases of hepatitis, mononucleosis and disease secondary to streptococcal infection on the other hand, a normal $\beta_2$fraction was found together with a distinct rise of the γ fraction. Generally however an increase of the γ fraction was accompanied by a corresponding change of the β fraction. Gall-stone was the disease most regularly associated with an increase of the β fraction, irrespectively of the γ value.

A *decrease of the albumin* was noted in all types of infections. As a rule, all severe infections irrespective of their cause were accompanied by a marked decrease of the albumin such as severe cases of bacterial infection and hepatitis (Table 12) In virus infections the albumin decrease was as a rule, relatively mild, also in most of the cases of hepatitis. In some cases of severe poliomyelitis in the acute stage with several days high fever the serum albumin as well as the protein pattern as a whole was almost normal. Primary atypical pneumonia was almost regularly associated with a distinct decrease of the albumin.

In acute infections *the total protein* was most frequently decreased, but in the later course of hepatitis, mononucleosis and toxoplasmosis it was often somewhat increased. Broadly speaking the total protein showed the same change in acute infection, as the albumin, but less marked This parallelism was closest in those infections in which the α-increase was relatively large compared with the γ increase.

DIFFERENT REACTION PATTERNS IN ACUTE INFECTIOUS DISEASES

The patterns of disorders fell largely into a few types. Table 14 shows the following 4 main types, namely that

Table 14 *Different patterns of changes in acute infectious diseases*

	Fever	Polymorphs	Alpha's	Gamma's
Acute bacterial infection type bacterial pneumonia	++	++	++	= or +
Viral infection				
type hepatitis	+	=	=	++
type viral meningitis	++	=	=	=
Primary atypical pneum nia	++	=	++	= or (+)

= largely no change
\+ increase

for bacterial pneumonia hepatitis, vi rus encephalo-meningitis and primary atypical pneumonia

Bacterial Pneumonia The pattern for bacterial pneumonia is characteri stic of most bacterial infections an intense primary inflammatory reac tion with fever leucocytosis increase of the α fractions and fibrinogen and in the more protracted and severe cases an increase of the γ fraction and the β fraction.

Virus Hepatitis The pattern of he patitis with relatively slight primary inflammatory reaction, but a substan tial and early increase of the γ frac tion, was also found in mononucleosis and in many cases of rubella and toxoplasmosis. These infections often showed polymorphopenia and relative or absolute lymphocytosis and often enlargement of the spleen and lymph nodes. Some bacterial infections such as typhoid, paratyphoid fever and sub- acute bacterial endocarditis showed a picture to some extent resembling that of hepatitis.

Virus Encephalo-meningitis. The third type of pattern—that of virus en cephalo-meningitis—showed marked fever but only slight changes in the other components of the primary re- action and as a rule, no change in the γ fraction but often an elevation of the specific antibody titer The exan thematous viral diseases of childhood morbilli and varicella resemble this group with slight disturbances.

Primary Atypical Pneumonia The most common sort of virus pneu monia is not caused by viruses. The cases studied showed only slight and late rise in the number of polymor phonuclear cells, but otherwise an equally intense primary reaction as bacterial pneumonia and many of them also a moderate increase of the β and γ fractions. Supervening bac terial infection may be partly re- sponsible for the changes in the later course

Virus infections with involvement of the mucosae, such as influenza and aphtous stomatitis, showed a similar picture which also in part may be caused by added bacterial infection.

Chapter IV

STATISTICAL ANALYSIS

PART I

GENERAL CONSIDERATIONS AND PLAN

General considerations As mentioned in Chapter III the selection of this material may not be quite representative of the diseases studied. The duration and particularly the natural history of a disease before admission are not properly known. Also after admission the follow up was not so systematic as might have been desired and was often discontinued before normalization of the features studied. The methodological errors of the values noted were often considerable.

The material is not very suitable for statistical analysis, which therefore cannot be expected to give accurate results. On the other hand, the positive correlations found are probably true for two reasons. Firstly substantial errors of the primary data tend to diminish the absolute value of the correlation coefficient found, and secondly the elimination of the mildest cases, and in some groups also of the most severe (vide page 16) tend to make a true positive correlation less apparent. For the same reason, however a low correlation coefficient obtained does not exclude the possibility of a true positive correlation to be present, and a markedly negative correlation coefficient may be found even in the absence of a true negative correlation.

The probability of the results has to some extent been strengthened by the efforts made to compensate for differences in the time table of the correlated features and by the agreement found between relations based on different measures of one and the same feature.

Finally of course positive correlations found are not equivalent to causal relationship.

Plan. The data were analysed statistically in an attempt to evaluate the relationship if any between

1) the different components of the primary inflammatory reaction,
2) the serum γ fraction value and other findings,
3) the serum β and β_1 fraction values and other findings,
4) the serum bilirubin value and other findings,
5) the serum albumin value and other findings.

PART II

METHODS

In the statistical analysis attempts were made to ascertain

1) the time relationship between the changes,
2) the quantitative relationship between approximately simultaneous findings in certain phases of the disease,
3) the interrelationship between the reduced indices of the reactants (for explanation *vide infra* section 3)
4) the relationship between the approximately maximal changes of some reactants and the reduced indices of others,
5) the interrelationship between the approximately maximal changes of some reactants
6) the interrelationship of various features studied by χ-analysis.

1. Analysis of time relationship between changes

The time relationship between the different changes in the course of bacterial infections and virus infections as well as in cardiac infarction was studied by means of scatter diagrams and mean curves given in part III of the previous chapter. These curves, however, showed mainly the regression of the different diseases. The time relationship of the changes in the initial progressive stage of disease is given by individual curves and mean curves for the first 3 days of acute bacterial disease and after endotoxin administration (Figs. 35—37).

2. Analysis of the quantitative relationship between approximately simultaneous findings

Pairs of different factors were studied for any correlation in certain phases of the disease, namely at the height of fever—first value noted after admission—and on return of temperature to normal—first value noted during first week of freedom from fever—and in the later afebrile regression—during the second to third afebrile week. In the analysis of cases of induced fever the original values noted before injection of endotoxin were used, but not any values in the second or third fever free week afterwards. The comparison was thus based on at most 3 recordings of each feature in a case studied.

The values compared were noted on the same day as far as reactants with the same time table are concerned, such as the temperature and the number of white blood cells. However, on

comparison between the temperature and serum α fractions, which react less promptly than the temperature attempts were made roughly to compensate for the lag of the change in the α globulins by comparing their values with temperature noted 48 hours previously. In a corresponding way an interval was used for comparison between some different types of reactants—see text under tables and figures.

On comparison between body temperature and other factors, the values for the latter were arranged in classes according to degree of fever and as 2 further classes the first afebrile and 2nd—3rd afebrile weeks respectively. The correlation coefficients found provide a rough measure of the relationship between the fever and other reaction.

In the statistical analysis of the relation between the protein changes—except CRP—and other changes in acute infection, data on cases of transient bacterial infection with fever for at most 3 days were excluded. As mentioned for pneumonia (page 29) and for induced fever (page 92) a very short fever is followed by only slight changes in the α-fractions and fibrinogen compared with cases of somewhat longer disease with about the same degree of fever, leucocytosis and amount of CRP in the serum.

Thus, at most 3 values for each feature were derived from one and the same subject and the lag of the change in the proteins was to a certain extent compensated and finally data on bacterial infections with fever for less than 4 days were excluded from the analysis.

3. Analysis of the relationship between the reduced indices of the reactants

An attempt was made numerically to estimate the total reaction in infection. The *index* of different factors was estimated by recording planimetrically in absolute values their deviation from the normal mean during the first 20 days of the disease, *i.e.* the main period covered by the primary inflammatory reaction. The index divided by 20 gave the mean deviation for this period and is referred to as the *reduced index* of the factor concerned (vide KEIDERLING et al. 1953) and used as a measure of its total change.

It was difficult to estimate these indices. As to the body temperature, daily recordings were sometimes available during the whole course while data on other factors were available only after admission to hospital. With our knowledge of the time table of the different reactants, in cases with a continuous disease from the onset of the disease to admission into hospital the temperature and W. B. C. were regarded as being the same from the 3rd day of the disease as the first value noted after admission. In the same way the CRP was assumed to be the same from the 4th day of the disease and the α fractions, fibrinogen and albumin values to be the same from the 5th day as the first values noted after admission. The serum bilirubin was assumed to be 2.0 mg./100 ml. on the day when jaundice was detected. The total change for the 20 days was cal-

culated on the basis of at least 3 data the first noted at the latest on the 10th day

4 Analysis of the relationship between the approximately maximal changes of some reactants and the reduced indices of others

The relationship between the gradual increase of the γ and β-fractions and the prompt primary inflammatory reaction in acute disease was studied in the following way The approximately maximal values of the γ and β-fractions were correlated with the reduced indices of various primary reactants. As *"approximately maximal" values* for the γ and β-fractions their levels were recorded during that phase of disease when they are generally known to reach their maxima *i.e* the γ value noted during the 15th to 30th day of disease and the β-values noted during the 11th to 30th day The mean of at most 3 recordings during these periods was used.

In biliary disease and hepatitis the γ and β-values noted during the 11th to 30th day of disease were correlated in the same way with the reduced index of serum bilirubin.

5 Analysis of the relationship between the approximately maximal changes of some reactants

In prolonged diseases, such as rheumatic fever and pulmonary tuberculosis, it was not possible to calculate any reduced index reflecting the total primary inflammatory reaction. In diseases of this type the *maximum γ-value noted was correlated with the maximum value for the α_2 fraction* the increase of which is the most long lasting component of the primary reaction For comparison maximal values for the γ and the α_2 fractions noted in the pneumonia series were correlated in the same way

In biliary disease due to gall stone, as a rule each case was examined electrophoretically only once In this disease the relationship was studied between the γ *and α_2 fraction values noted in the third week of the disease* when the increase in the α_2 was usually still pronounced and when the increase of the γ fraction—when present—had usually had time to become distinct. The same relationship was studied in bacterial pneumonia

In biliary disease due to gall stone the following findings were collected at the height of the disease namely serum bilirubin, γ and β-fraction values noted on one and the same day during the 2nd to 4th week of disease and the mean temperature during the week preceding these determinations. These *approximately simultaneous and approximately maximal values* were then intercorrelated.

6 Interrelationship of various features studied by χ^2-analysis

The maximal changes noted for the cases in a series of *infectious mononucleosis* and *infectious hepatitis* respectively were studied in the following way

Generally maximal changes noted of various features were analysed. In mononucleosis the mean curves drawn for data for the fibrinogen, haptoglobin and the

number of polymorphonuclear cells had a diphasic course with an initial slight i crease during the febrile stage and then a phase of subnormal values. Recordings were made of the highest lue noted for these 3 phenomena during the first 2 weeks of the disease and of the lowest recorded d ring the later course

In hepatiti the alkaline phosphatase α and β fraction howed n distinct peak. For these the mean value f the 3 highest recordings were used when possible

In the χ analysis for the two types of disease the youngest and the oldest eighth of the series were eliminated The values for each of the features in the remaining 75 % of the series were primarily divided into two approxi mately equal groups of values above and below the median value. Pairs of the different phenomena were correlated in 2×2 tables, in which the num ber of cases of each phenomenon with a low and high value, respectively were noted.

The frequencies obtained were then submitted to analysis in the follow ing way If the number observed in each quarter is called a, b c and d, the expected number E of the quarter which corresponds to a is calculated by the formula

$$E = \frac{(a+b)\times(a+c)}{a+b+c+d} = \frac{R\times M}{N}$$

The standard error for this expected value is

$$\sqrt{E\times\frac{N-R}{N}\times\frac{N-M}{N}}$$

As stated, the material in the tables was divided into two approximately equal groups for each phenomenon, *i e* a+b=N/2 and a+c=N/2, approximately The expected number in each quarter will then be approximately the same N/4 with the standard error $1/4\sqrt{N}$

An expression for the relation between the two phenomena in a field of the 2×2 table will then be the difference between observed and ex pected number of cases divided by the standard error or

$$\frac{O-E}{\frac{\sqrt{N}}{4}}$$

This quotient was calculated for all the pairs of phenomena analysed in 2×2 tables. For each 2×2 table with the above-mentioned approxima tion $\chi = \frac{4\ (O-E)}{\sqrt{N}}$ or the square of the above mentioned quotient.

The degree of freedom for each 2×2 table is one. The square root of the value found in the χ distribu tion for one degree of freedom charac terizes P for the above mentioned quotient Thus P=0 001 with the quotient 3.3 P=0 01 with the quotient 2 6 P=0 05 with the quotient 2.0 and P=0 10 with the quotient 1 6.

The correlations suggested by the χ analysis between the maximum val ues of the different phenomena as well as their time relations, were tested by plotting in a co-ordinating system

The part of the material which fell outside the χ analysis, the *lowest and the highest age classes* in mononucleosis also P B negative cases and jaundiced cases, was arranged in groups of increasing degree of each disorder In

this way the average deviation of these cases from the χ analysed part of the material was demonstrated

Finally in the individual patients with mononucleosis the relation between the sum of the following primary inflammatory reactants α_1 α fraction, fibrinogen, CRP and tonsils and the sum of the following secondary reactants γ-fraction, length of spleen and absolute number of mononuclear cells in the blood, was examined in the following way For each of these phenomena the material was divided into 4 equal groups—graded 1—4 points—of increasing severity of the disorders. For each individual these points expressing the degree of disorder were added up to *one sum showing the primary reaction and one sum showing the secondary reaction* These two scores were correlated.

RESULTS

1 Time relationship between different changes

The time relationship between disorders in various infectious diseases and in myocardial infarction is given in Figs. 3—37

The almost invariable *fever* generally culminated within the first few days of the disease. This also holds for the increase in the number of *polymorphonuclear cells* in bacterial infections, in which both changes were often promptly controlled by antibiotics. The fever in the virus infections usually regressed within about a week, though often much slower in mononucleosis.

In injury of short duration, such as that produced on administration of pyrogens, the body temperature and the number of polymorphonuclear cells reached their maxima already within 6—12 hours, the *CRP* within about 1 day and the total *α fractions the haptoglobin and the fibrinogen* within 5—6 days, and with a more marked peak for the pure substances such as the latter two, than for mixtures of different substances such as the two α fractions.

After such brief injury of a few hours duration the fever and leucocytosis disappeared within a day or two. The CRP disappeared some days later the α_1 fraction became normal after about one week, the haptoglobin and the fibrinogen seem to have required 1 to 2 weeks before they became normal and the total α_2 fraction at least 2 weeks.

The change in the *γ fraction* in infectious diseases differed in time from that of the primary inflammatory reaction. In bacterial infection the γ increase started about 1 week after onset of disease and generally reached its maximum in the 3rd to 4th week, after which it regressed slowly to reach normal a few weeks at most, after the α fraction and the albumin fraction. In virus hepatitis and mononucleosis the γ fraction was most often increased already at the first examination. It regressed very slowly and persisted as the only electrophoretic abnormality often for a long time

Many but not all, of the mean curves for the *antibody* titers resembled that for the γ fraction. Thus, the mean curves for antistreptolysin O titer and γ fraction in different types of streptococcal disease were largely parallel, and the median curves for both Widal H and O followed closely the mean

curve for γ fraction in salmonellosis of the typhoid type. In toxoplasmosis, on the other hand the curves for the dye test titer and particularly the curve for complement fixation titer seemed to ascend slower than the curve for the γ fraction. In individual cases of different infections there was no regular correlation in time between antibody titer and γ fraction.

The mean and individual *thymol turbidity values* noted in hepatitis and mononucleosis changed roughly parallel to the γ fraction values (Figs. 18 and 22) During the first and second weeks of mononucleosis, however the thymol turbidity values increased relatively less than the values found for the γ fraction.

In mononucleosis the relative *lymphocytosis* which persisted for a long time in the later course, was likewise related in time to the γ fraction—this holds for the mean curves and to a certain extent also for the individual values (Fig 22) The slight relative lymphocytosis, which occurred in hepatitis and in the later course of severe bacterial infections showed no distinct relationship with the γ increase.

The mean curves for fibrinogen and haptoglobin in mononucleosis changed roughly inversely to the course of γ fraction (Fig 22)

As a rule *the two β-fractions* showed only slight changes so that their time relationship with other features was more difficult to assess. Generally the β_1 fraction, when increased, reached a maximum somewhat before or at the same time as the γ fraction. Their curves ran a parallel course, but the β fraction regularly became normal before the γ fraction.

The β fraction increased still more slowly as a rule, than the γ fraction, often with a flat maximum as late as one month after the onset but it generally reached the normal mean before the γ fraction The mean curve for the β fraction was not distinctly correlated in time with those for other phenomena

The decrease in the *albumin* following brief injury such as the effect of pyrogen, occurred within 1 to 2 days and roughly at the same time as the α fractions began to increase the greatest decrease was 2 to 4 days after the injury

In bacterial infection the albumin minimum coincided with the α_1 maximum and both became normal at roughly the same time. In hepatitis and mononucleosis the albumin minimum coincided in time with the height of the γ increase, but in these diseases the albumin became normal earlier than the γ fraction.

2. Quantitative relationship between various components of the primary inflammatory reaction

The relationship between approximately simultaneous values of various components in certain phases of disease is given in Tables 15—17 and in Figs. 38—40

The *body temperature* (Table 15 and Fig 38, 39 and 40) was positively correlated with the number of polymorphonuclear cells, with the CRP and both the α fractions in bacterial diseases, and was weakly correlated

Table 15 *Relatio between b dy temperature and oth r component f the primary inflammat ry reaction in vari u di ease*

Correlation of *temperature* with	Pol morphs	CRP		α_2	Fibrinogen	Haptoglobi
Bacterial pneumonia	+ 0.63 44 (85)	+ 0.83 n 43 (93)	r + 0.76 42 (81)	+ 0.77 42 (81)	+ 0.63 n 31 (51)	+ 0.73 13 (26) (incl. 5 cases of ton illitis)
Biliary disease with jaundice	+ 0.62 52 (93)	—	+ 0.52 56 (61)	+ 0.49 50 (61)		
Disease sec. t treptococc. inf	+ 0.35 20 (45)	r + 0.56 21 (28)	+ 0.53 29 (41)	+ 0.52 n 29 (41)		
Mononucleosis	+ 0.36 80 (153)	+ 0.61 76 (112)	r + 0.48 79 (143)	+ 0.41 79 (143)		
Hepatitis	0	+ 0.43 47 (86)	+ 0.38 45 (111)	0		
Viral meningitis	+ 0.34 44 (86)	0	0	+ 0.33 n 37 (61)		

Correlation bet een temperature and *polymorph* based on alues ted on the same day
CRP based alues for CRP on the 1 t day fter the alues for temperature
other *proteins* based on values f the latter on the 2nd day after the alues for t mperat re.

< ± 0.20 with > 20, noted as 0.
= Number of individuals observed
V lues in bra kets = Number of observations
V lue for correlation coefficient corresponding to P 0.05 and 0.02, respectively with increasing number of indi idual observed:

	P 0.05	P 0.02
5	0.75	0.83
10	0.56	0.66
15	0.48	0.56
20	0.42	0.49
25	0.35	0.41
30	0.30	0.36

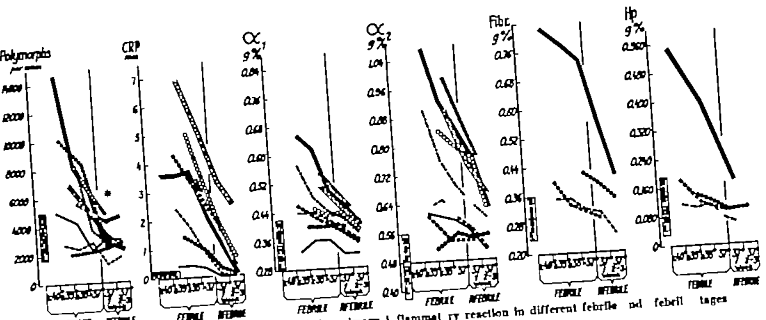


Fig 38. Mea alues for arious components of the primary i flammat ry reaction in different febrile nd febril tages of various diseases.

Polymorphs in cases of myocardial inf rction nd p lmonary t berculosis calculated as 2/3 of W B.C.

Fig. 39 Correlation in bacterial pneumonia between body temperature and the α-fractions.
○ values of cases with fever ≤ 3 days.
● > 3 days.
Correlation based on alues for the fractions, noted 48 hours after alues for the temperature.

with the CRP and the α_1 fraction in mononucleosis and hepatitis But the correlation in pneumonia with the α fractions was only evident when data on cases with very short fever were excluded (Fig 39) The temperature was positively correlated with the fibrinogen and haptoglobin in pneumonia. In virus meningitis no relationship was found between the temperature and the polymorphonuclear cells and the protein components, which did not change very much.

Also after induced fever and, to some extent, in biliary disease with jaundice the increase of both α fractions was smaller on the average than in bacterial pneumonia with a corresponding fever

The correlation of the number of *polymorphonuclear cells* in the blood (Table 16 a and Fig. 41) with the inflammatory protein reactants was only weakly positive or questionably positive in conditions caused by bacteria. And no distinct relationship was found in virus infections.

However a positive correlation between cellular and protein components of the primary inflammatory reaction was more evident in the experiments with induced fever

CRP mm

FEBRILE AFEBRILE

Bact. pneumonia

Hepatitis

Fig. 40 Correlation i bacterial pneumonia and infectious hepatitis, respectively between body temperature and CRP Correlation based on values, noted sim ltaneously f r both.

Fig. 41 Correlation in bacterial pneumonia between the number of polymorphonuclear cell in the blood nd the α-fractions.

○ values of cases with fever ≤ 3 days.

● > 3 days.

Correlation based on alues for the fractions, n ted 24—72 hours after values for the polymorphs

T ble 16 a. Relation between values f r polymorphs and other components f primary inflammat ry reacti n in vari us diseases

Correlation of *Polymorphs*, bbrev "Poly (1000 per cu mm) with	CRP (mm)	α (g‰)	α (g‰)	Fibrinogen (g)	Haptoglobin (g‰)
Bacterial pneumonia	+ 0.58 43 (71) Poly = 2 1 CRP + 2 0	+ 0 48 31 (13) Poly = 21 α_1 — 4 7	+ 0 60 31 (43) Poly = 14 α_2 — 8 0	r + 0 17 16 (23) Poly = 8 8 F + 1 4	r + 0 73 n 16 (70) P ly = 17.5 Hp — 1 4
Biliary disease with jaundice	—	+ 0 83 13 Poly = 27 α_1 — 8 8	+ 0.73 n 13 Poly = 11 α_2 — 5 6	—	—
Disease sec t streptococc. inf	r + 0.52 20 (21) Poly = 0 9 CRP + 3 0	—	—	—	—
Fever induced by endot xi	+ 0 81 8 (16) Poly = 1 8 CRI + 2.1	+ 0 72 6 (15) 1 ly = 57 — 18	+ 0 65 6 (15) Poly = 30 α_2 — 15.7	Fig. 12	Fig. 42
Mononucleosis	r + 0.45 61 (77) Poly = 0 16 CRP + 2.2	—	—	0	0
Hepatiti	0	0	0	—	—
Viral meningiti	0	0	0	—	—

Correl ti between *polymorphs* nd *CRP* based on l es noted on the same l y
other *proteins* based on lues for the l tter on the 1 t 3rd d y fter the lues for polymorphs.

< ± 0 20, with n > 20, noted 0.

=N mbe of indi idual bserved. Values in bracket =Number of observ tions.

V lue for correlation coefficient correspondi g t P 0 05 nd 0 02, respectively with increasing number of indi idual observed: see Table 15.

Incl. 5 cases of other kind of bacterial infection.

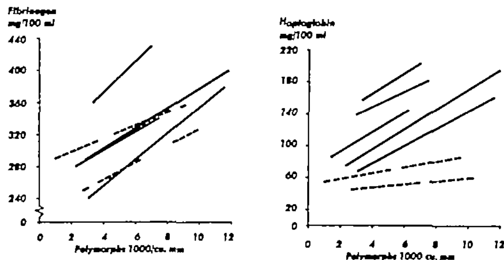


Fig. 42. *Relati nship between the hanges f polymo phs and of fibrinogen and haptoglobin in feve induced by endotoxin.*

Data on polymorphs and fibrinogen (in 6 indi iduals) and on polymorphs and haptoglobin (in 7 individual) noted in afebril stat before administration of endotoxin and connected by lines of regression with data corresponding t maximal fever after the dministration. The latter values, for polymorphs at the height of fever and for the proteins 2 days later

_____ *Lines of regression for individuals treated with pyrifer*

Lines of regression for individuals treated with pyrexal.

Table 16 b. *Relati nship between changes of polymorphs with changes of haptoglobin and fibrinogen in fever induced by endotoxin*

Mean differences (and ranges) between original values and values noted, for polymorphs t the height of fever for haptoglobin and fibrinogen 2 days later and the quotients between the sum f the differences.

1st value noted for polymorphs		Poly
2nd		Poly
1st	haptoglobl	Hp_1
2nd		Hp_2
1st	fibrinogen.	Fibr
2nd		Fibr

m Poly —Poly (7) 6 700/cu.mm (rang 3, 00—9,500

m Hp_1—Hp_2 (7) 58 mg/100 ml (range 15—120)

m Fibr —Fibr (6) 88 mg/100 ml (range 50—140)

$$\frac{\Sigma(\text{Poly} - \text{Poly}_2)}{\Sigma(\text{Hp}_1 - \text{Hp}_2)} = 116$$

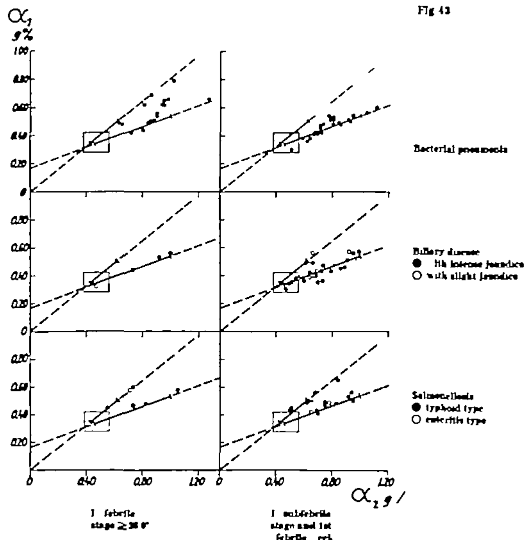
$$\frac{\Sigma(\text{Poly} - \text{Poly}_2)}{\Sigma(\text{Fibr} - \text{Fibr}_2)} = 80$$

Tabl 17 *R latio between serum fracti n and other components of primary inflammat ry reacti n in various diseases*

Correlation of fracti (g%) with	α_2/β (g)	Fibrinogen/pl (g)	Haptoglobin/s (g)
Bacterial pneumonia	Fig 13	+ 0 62 31 (53) α_1 = 0.26 F + 0.22	+ 0 00 10 (23) α_1 = 0 38 Hp + 0.22
Bacterial t nsillitis	—	r + 0. 6 n 18 (31) = 0.11 F + 0 21	—
Salmonellosis	Fig 43	Fig. 44	—
Biliary disease with jaundice	Fig. 43	Fig 44	—
l lmonary tuberculosis	See t xt α_1 = 0 37 α_2 + 0 17	—	—
Fever induced by endot xin	See text α_1 = 0 81 α_2 + 0	r + 0 63 0 (14) α_1 = 0 50 F + 0.21	—
Atypical pneumonia	—	r + 0.67 n 10 (19) = 0.35 F + 0.26	+ 0 80 8 (12) α_1 = 0 43 Hp + 0 33
Mononucleosis	Fig. 43	Fig. 44	—
Hepatitis	Fig. 43	Fig. 44	—
Viral meningitis	Fig. 43	—	—

Correlations re based on values from the same day
= Number of indi iduals observed.
Values in bracket = Number f observations.
Value for correlation coefficient corresponding t P 0 05 and 0 02, respecti ely with increasing mber f individu l bserved; see Table 18.

incl 5 cases of tonsillitis.



Data corresponding to the afebrile stage before, and maximal fever after the *administration of endotoxin* gave similar lines of regression for polymorphs on fibrinogen and haptoglobin in all individuals studied (Fig 4? and Table 16 b based on values noted before injection of endotoxin and on values, for polymorphs at the height of fever and for the proteins 2 days later) While no relationship is seen between data noted before the administration of endotoxin, a positive correlation is apparent between the increase in the number of polymorphs and the increases of haptoglobin and fibrinogen following the injection of endotoxin.

The increase of fibrinogen, and particularly haptoglobin, in 2 individuals was less than in the others. These 2 had received pyrexal that ha a sh rter action

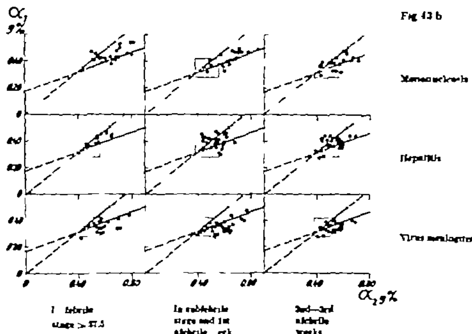


Fig 43 and b. Relatio in arious infections between the α_1 and α_2-fractions in different stages of the disease.
Steep line in the field Regression line of α_1 on α_2 in the progressive stage of protein changes following endotoxin administration.
Flat line Regression line of α_2 on α_1 in cases of healing pulmonary tuberculosis. Unbroken part of the lines correspond t the range of values from which the regres ions re cal culated.

than pyrifer which was gi en to others their haptoglobin alues were also somewhat low initially possibly reflecting a more rapid haemoglobin turnover than in the others.

The *CRP-content* of serum was found to be positively correlated with the severity of the changes of α frac tions and fibrinogen in bacterial infec tions, but not distinctly in virus infec tions

Simultaneous values for the *two α-fractions* proved to be positively cor related in bacterial conditions, atypical pneumonia, influenza and in myocardial infarction, but less distinctly in hepatitis, mononucleosis and virus meningitis

The regression of α_1 on α_2 was not the same during the entire course of the primary inflammatory reaction. The regression, or rather the progression in the progressive initial phase of the protein changes during the first few days after endotoxin injection, was calculated by means of a covariance analysis from data on 3 subjects. The regression in the regressive phase of disease was calculated in the same way from data on 3 patients

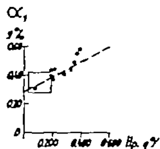


Fig. 44 Correlation between the serum α_1-fraction and haptoglobin in salmonellosis. The lin of re gression of α_1 on haptoglobin in acut pneumonia (NYMAN 1959) drawn.
● cases of typhoid type
○ enteritis type

with slowly healing lung tuberculosis The 3 cases in each group showed practically identical regression coef ficients, namely 0.81 for the progres sive stage and 0.37 for the regressive stage. The corresponding values for the regression lines are given in Table 17

Data on α fractions noted in fever and during the later subfebrile and afebrile stage respectively of various diseases are plotted in Fig 43 In which also the above mentioned re gression lines are inserted.

On abatement of the fever bacterial pneumonia showed an α/α quotient corresponding to that for the cases of healing tuberculosis. During the pre ceding stage with evident fever the quotient was usually higher approach ing the quotient in the progressive stage of the induced fever cases. In biliary disease with jaundice the pic ture was largely the same. In salmo- nellosis both of typhoid and enteric type the α/α_1 quotient was relatively high during the entire course as well as in hepatitis and mononucleosis On the other hand, the cases of virus meningitis showed a low α/α_1 quo- tient even during the febrile stage.

The change of the α_1 fraction is mainly a manifestation of analogous change in the *haptoglobin* (JAYLE et al. 1955) As expected from NYMAN s report (1959) in the present series of bacterial as well as of atypical pneu monia a strong positive correlation was found between α_1 and haptoglobin with a roughly identical regression line for α_1 on haptoglobin in both types of disease (Table 17) On the other hand in the present series of hepatitis and mononucleosis, which included some of NYMAN s cases, and of salmonellosis (Fig. 44) no apparent correlation was found between serum α_1 fraction and haptoglobin. The va lues in these diseases for the latter protein were most often low compar ed with those given by the regression line of α_1 on haptoglobin in acute in fections (NYMAN)—for discussion see page 140

In tonsillitis and atypical pneumo- nia the *fibrinogen* was positively cor related with the α_1 *fraction* (Fig. 45 and Table 17) In bacterial pneumonia some of the fibrinogen values noted were relatively low compared with

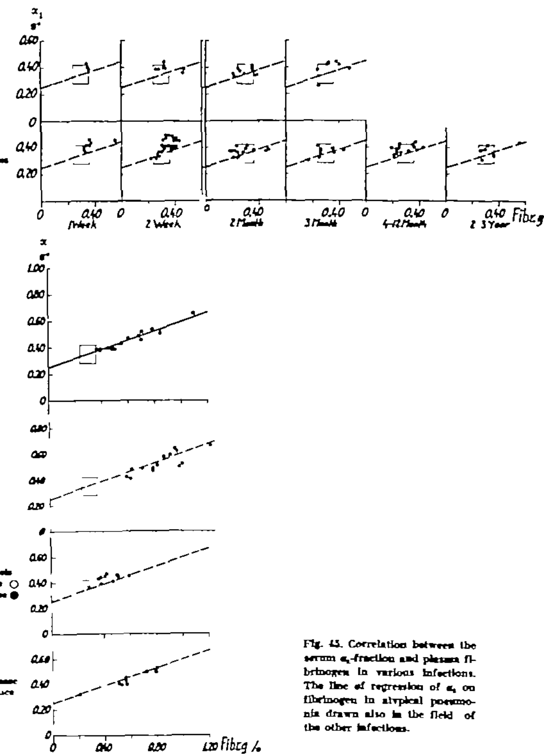


Fig. 45. Correlation between the serum α_1-fraction and plasma fibrinogen in various infections. The line of regression of α_1 on fibrinogen in atypical pneumonia drawn also in the field of the other infections.

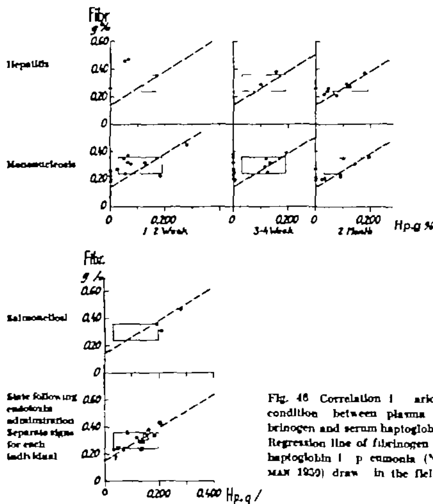


Fig. 46 Correlation i various condition between plasma fibrinogen and serum haptoglobin. Regression line of fibrinogen on haptoglobin i p eumonia (NYMAN 1959) draw in the fields.

the regression line for α_1 on fibrinogen in atypical pneumonia. Particularly in mononucleosis, hepatitis and sal monellosis the fibrinogen was relatively low The level was low for one year after the disease in some cases of mononucleosis (Fig 45)

NYMAN found a strong positive cor relation between *fibrinogen* and haptoglobin in pneumonia. Also after endotoxin administration (Fig. 46) a posi tive correlation was found between haptoglobin and fibrinogen. In 2 subjects with an initially low haptoglobin value the increase of haptoglobin was low relative to both the increase of the fibrinogen and the polymorphs. Hepa titis, mononucleosis and salmonellosis showed no distinct correlation be tween the two proteins (Fig 46)

Relationship between *the reduced indices of primary inflammatory re actants* are studied in various kinds of infection and in myocardial infarction.

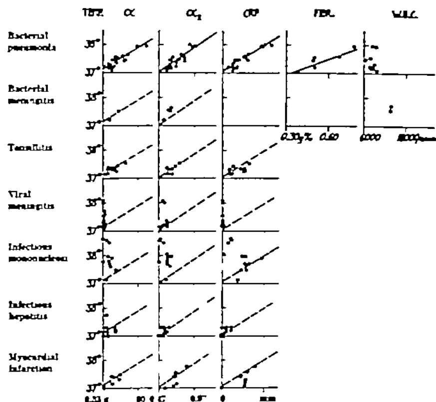


Fig. 47 Relation between the reduced indices of *body t mperature* and other components f the primary inflammatory reaction in various diseases. The line of regression for temper ture on the other reactants, in bacterial pneumonia, is drawn also in the fields of the other diseases.

Table 18 and Fig 47 show this rela tionship between the reduced index of *temperature* on one hand, and on the other the index of the CRP the α_1 and α_2 fractions in acute bacterial pneu monia, bacterial meningitis, tonsillitis, virus meningitis, mononucleosis, he patitis and in myocardial infarction. They also give the relationship between temperature and fibrinogen in pneumonia and between temperature and W B C. in pneumonia and in bac terial meningitis.

In bacterial pneumonia a significant positive correlation was found between the body temperature and other primary inflammatory reactants with the exception of the number of white blood cells.

The number f cells fluctuated mar kedly i the same patient from day to day with therwise roughly the same clinical picture, so that the calculation f the re duced index of the W B C. from few counts during the course was inaccurate. It i also possible that a few of the pa tients with diagnosis of bacterial pneu-

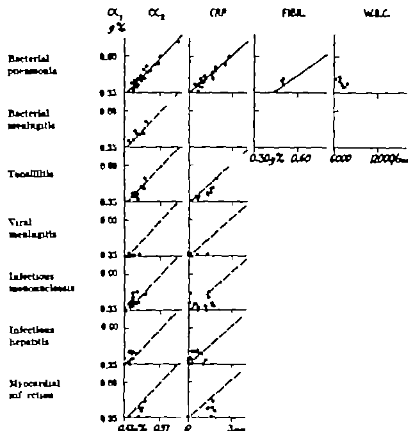


Fig. 48. Relation between the reduced indices f the serum fraction and other primary inflammatory reactants in arious diseases. The line of regression of α_1 on the ther reactants, in bacterial pneumonia, is drawn also in the field of the other diseases.

monia had in reality an atypical pneumonia in spite of a negative cold agglutina tion test.

In order to facilitate comparison, the regression line for temperature on other reactants in bacterial pneumonia, is also inserted in the fields for other infections (Fig 47)

In other bacterial infections the tem perature was also positively correlated with other reactants This also holds for cardiac infarction In bacterial meningitis the relatively high degree of fever is striking

In virus meningitis and mononucle osis the fever was high compared with the slight change of the other reac tants. No evident correlation was found between the temperature and other primary inflammatory reactants.

Table 10 and Fig 48 show corresponding relations between the *reduced index of the α_1 fraction* on one hand, and the indices of other primary inflammatory reactants on the other

In bacterial pneumonia a positive correlation was found between the α fraction and all primary inflammatory

Table 18 *Relation between the reduced indices of body temperature and other components of th primary inflammatory reaction in various diseases*

Correlation of *temperature* with	a_1		CRP	Fibrinogen	W.B.C
Bacterial pneumonia	r +0.8[illegible] 21	+0.84 n 21	r +0.80 n 24	r +0.61 8	+0.42 n 15
Bacterial meningiti	+0.8[illegible]* 8	+0.91 ** n 8	—	—	r +0.51 12
Tonsillitis	+0.72* n 11	+0.26 n 11	r +0.49 n 10		
Viral meningiti	0 n 13	0 n 13	—		
Mononucleosi	r 0 n 20	0 n [illegible]0	r 0 n 16		
Hepatiti	0 14	+0.43 n 14	r +0.75 n 14		
Myocardial inf rction	+0.[illegible]0 n 9	+0.64 9	r +0.42 n 9		

r < ±0.20 noted as 0

Tabl 19 *Relation between the reduced indices of the a_1-fraction and other component f the primary inflammat ry reacti n in variou diseases*

Correlation of a_1 with	a_2	CRP	Fibrinogen	W.B.C.
Bacterial pneumonia	+0.94** 24	+0.8[illegible]** n 24	+0.[illegible]9 n 8	+0.[illegible] n 12
Bacterial meningitis	+0.81 8	—	—	0 8
Ton illitis	+0.80* 11	+0.73 n 10		
Viral meningitis	+0.34 12	+0.[illegible] n 12		
M nonucleosis	+0.73** 20	+0.[illegible] 16		
Hepatiti	+0.56 14	+0.48 n 14		
Myocardial inf rction	+0.84** 9	+0.73 n 9		

< ±0.20 is noted as 0.

Fig. 49 a

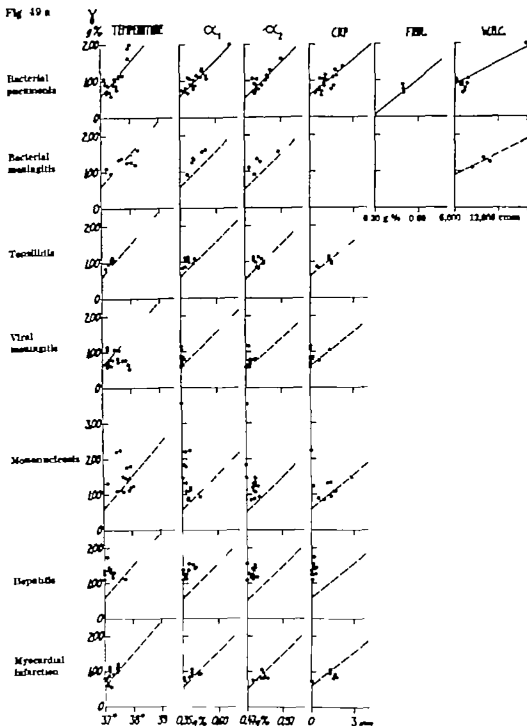
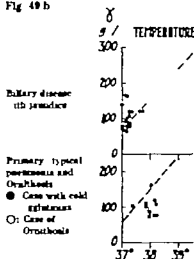


Fig. 49 and b. Relation between the *pproximately maximal level f the erum γ-fraction* in the 15th—30th day f disease and the reduced index of different primary inflammatory reactant in various diseases. The line of regression of γ on other reactants in bacterial pneumonia is drawn also in the fields of the ther diseases.



reactants except the number of white blood cells. The regression line for pneumonia is also inserted in the fields of the other infections.

Other bacterial infections and also hepatitis and mononucleosis showed a positive correlation between the a_1 and a_2 fractions but virus meningitis showed no correlation. This disease was followed by a relatively slight change in the a_1 fraction, while mononucleosis and hepatitis were followed by a relatively slight change in the a_2 fraction. In cardiac infarction a positive correlation was found between the a_1 and a_2 fractions the a_1 change was relatively slight as in virus meningitis.

3 R lationship between th γ-fraction and other feat res

Relationship between the *approximately maximal level of the γ fraction and the reduced indices of the primary inflammatory reactants* was studied in different kinds of infectious disease and in myocardial infarction (Table 20 and Fig 49)

In bacterial pneumonia the γ fraction showed a positive correlation with all primary inflammatory reactants, but the correlation with the number of W B C. was only weak. The regression lines of γ-fraction on the primary inflammatory reactants in bacterial pneumonia are also inserted in the fields for other diseases.

In the other bacterial infections largely the same correlations were found, though they were not always significant owing to the small number of cases. The values found for the γ fraction in tonsillitis and biliary disease were somewhat higher than what might be expected from the regression line of the γ fraction on temperature in bacterial pneumonia.

In the virus infections studied and atypical pneumonia, on the other hand, the level of the γ fraction showed no correlation with the primary inflammatory reactants. In virus meningitis and atypical pneumonia relatively low γ values were found in relation to the change in temperature, but in hepatitis relatively high γ values. In hepatitis and mononucleosis the increase of the γ fraction was also found to be considerable compared with the slight increase of the α-fractions and the CRP

In cardiac infarction the γ fraction showed no correlation with body temperature. The dispersion of the temperature values was, however also relatively slight. One could therefore not exclude the possibility of a correlation with the γ fraction in cases with

Table 20 *Relation between the level of the γ-fraction (during 15—30 day of disease) and the reduced indices of the components of the primary inflammatory reaction in various diseases*

Correlation of γ with.

	Temperature	α_1	α_2	CRP	Fibrinogen	W.B.C.
Bacterial pneumonia	+0.74 n 24	r +0.86** n 24	r +0.78 ** n 24	r +0.60 n 24	r +0.77 n 8	+0.62 n 15
Bacterial meningitis	r +0.82* n 12	+0.54 n 8	+0.70 n 8	—	—	+0.65 n 12
Tonsillitis	+0.62* n 11	r +0.80 n 11	r +0.55 n 11	+0.70* n 10		
Viral meningitis	0 n 20	+0.39 n 13	+0.41 n 13	+0.42 12		
Mononucleosis	+0.23 n 20	r 0 n 20	r 0 n 20	r 0 n 16		
Hepatitis	—0.20 n 14	+0.20 n 14	0 n 14	0 n 14		
Myocardial infarction	+0.21 n 20	+0.70* n 9	+0.77 n 9	+0.70 n 9		

$< \pm 0.20$ is noted as 0.

Table 21 *Relation between serum γ- and α_2 fraction maximal values (g %) and values noted in the 3rd week of disease respectively in various disease*

	Max. γ– Max. α_2	3rd week γ—3rd week α_2
Bacterial pneumonia	+0.46** n 46 $\gamma = 0.83\ \alpha_2 + 0.38$	+0.71** 25 $\gamma = 1.45\ \alpha_2 + 0.01$
Pulmonary tuberculosis	0.59* n 13 $\gamma = 0.88\ \alpha_2 + 0.49$	—
Disease secondary t streptococcal infection	+0.59* n 22 $\gamma = 1.21\ \alpha_2 + 0.46$	—
Gall-stone with jaundice	—	+0.71 n 16 $\gamma = 1.89\ \alpha_2 — 0.00$

a higher grade fever particularly since a weak positive correlation was found between the γ fraction on one hand, and the α fractions and CRP on the other hand.

The relationship between *the maxi mal levels of the γ fraction and of the α_2 fraction noted* was studied in cases of pulmonary tuberculosis, diseases secondary to streptococcal infection

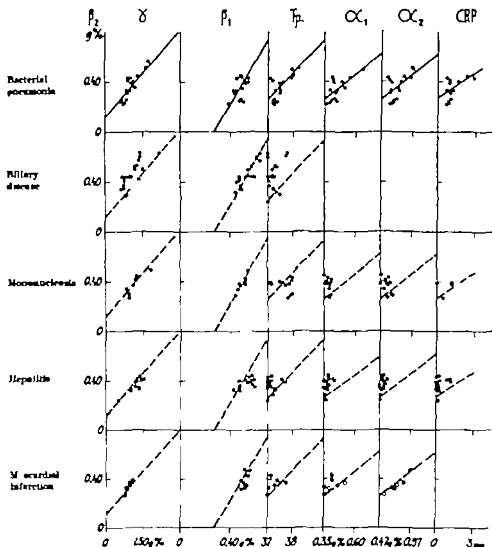


Fig. 50 Relation between the *approximately maximal level f the rum β-fracti n*, and γ-fractio m the 11th—30th day of disease, and the reduced index f primary inflammatory reactants in various diseases. The line of regression of β on the other features in bacterial pneumonia is drawn also in the fields of the other diseases.

● Values obtained from fresh sera

○ Values obtained from sera which have been kept in the frozen state.

and in bacterial pneumonia. All 3 types of disease showed a weak positive correlation (Table 21) The regression of the γ fraction on the α_1 fraction tended to be steeper in diseases secondary to streptococcal infection than in the other two groups.

The relationship between *the γ- and*

Table 22 *Relation between the level of serum β_1-fraction, and γ and β_1 fraction (during 11—30 day of disease) and the reduced indices of body temperature α-fractions and C R P in various diseases*

Correlation of β with

	Number of cases	γ/s	β_1/s	Temperature	α_1/s	α_2/s	CRP
Bacterial pneumonia	19	r + 0.86	+ 0.50*	r + 0 73**	+ 0 76***	+ 0.65**	0 71 **
Gall stone	23	r + 0 69*	+ 0.40	r + 0.27	—	—	
M mononucleosis	13	+ 0 63	+ 0.21	0	— 0 34	r — 0.41	
Hepatitis	17	r + 0 66	+ 0,54	0	+ 0 33	r + 0.56	
Myocardial infarction	9	+ 0.82*	+ 0 49				

Values for $< \pm 0\ 20$ noted as 0.

α_1 values noted during the 3rd week of the disease was studied in biliary disease with jaundice and in bacterial pneumonia (Table 21) A positive correlation was found in both diseases. The regression of the γ on the α_1 fraction was steeper in biliary disease than in pneumonia.

4 Relationship between the β-fractions and other features

The relationship between the approximately maximal value for *the β fraction* and corresponding values for the γ and β fractions and the reduced indices of primary inflammatory reactants in various diseases is apparent from Table 22 and Fig 50 In myocardial infarction only sera that had been kept in the frozen state for several weeks were used, so that the β values noted were lower than the true β level.

In all the diseases studied the β fraction showed a close correlation with the γ fraction and in bacterial pneumonia a positive correlation with all the primary inflammatory reactants and also with the β_1 fraction.

In biliary disease and in cardiac infarction the β value was generally higher but in hepatitis and mononucleosis lower than in bacterial pneumonia with a corresponding γ value.

Between approximately simultaneous and maximal values for *the β fraction* and temperature during the 2nd to 4th week of biliary disease a partial, negative correlation was found after elimination of the effect of the β values (Table 24)

Concerning the relationship between the β-fractions and some other features *vide infra*

5 Relationship between the serum bilirubin and other features

In hepatitis no correlation between *the reduced index of serum bilirubin* and other features was found, and in biliary disease an evident correlation only with the approximately max

Table 23. *Relati n between the reduced indice of temperature and serum bilirubin and approximately maximal value for β β and γ-fractions (during 11—30 day of disease) in 25 cases / biliary disease due to gall stone*

Temp Bilirubin − 0.39	Tem β 0	emp β + 0.27	Temp γ + 0.49
T mp Bilirubin γ − 0.64**		em β_1 Bilirubin + 0 44	Temp γ Bilirubin + 0 69**
	Bilirubin β + 0 60	Bilirubin β_1 + 0.30	Bilirubin γ + 0 30
	Bilirubin β emp + 0.60**	Bilirubin β_1 Temp + 0 46	Bilirubin Temp + 0 61**
		β_1 β_1 + 0 40	β γ + 0.36
			β_1 + 0.69***

Corre ponding elationsh p in 17 case / infectious hepatitis

Bilirubin Temp − 0.36	Bilirubin β 0	Bilirubin β_1 0	Bilirubin 0

Explanations. The correlation coefficient bet een 1 and 2.
The partial correlation coefficient between 1 and 2 after elimination of the effect of 3.

Values for $< \pm 0\ 20$ noted as 0

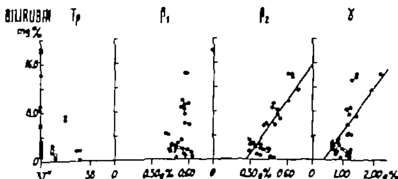


Fig. 51 R lation between *pproximately simultaneous and maximal val for erum bili bin* and for serum β- and γ-fractions and for temperature (mean temperature during the preceding week) *in biliary disease*

● Case with mean temperature ≥ 37.5

O: Case with mean temperature < 37.5

Table 24 Relation between approximately simultaneous and maximal values for serum bilirubin β_1, β_2 and γ-fractions (values from the same day during 2nd to 4th week of disease) and temperature (the mean during the week preceding the electrophoretic examination) in 41 cases of biliary disease due to gall-stone)

$r_{Temp\ bilirubin}$ 0	$r_{Temp\ \beta_1}$ −0.31	$r_{Temp\ \beta_2}$ +0.30	$r_{Temp\ \gamma}$ +0.55***
$r_{Temp\ bilirubin\cdot\gamma}$ −0.26	$r_{Temp\ \beta_1\cdot\beta_2}$ −0.34	$r_{Temp\ \beta_2\cdot\beta_1}$ +0.53**	$r_{Temp\ \gamma\cdot bilirubin}$ +0.65**
	$r_{bilirubin\ \beta_1}$ +0.37	$r_{bilirubin\ \beta_2}$ +0.66**	$r_{bilirubin\ \gamma}$ +0.53**
		$r_{\beta_1\beta_2}$ +0.48**	$r_{bilirubin\ \gamma\cdot Temp}$ +0.60**
			$r_{\beta_1\gamma}$ 0
			$r_{\beta_2\gamma}$ +0.58***

Explanations r The correlation coefficient between 1 and 2.

r The partial correlation coefficient between 1 and 2 after elimination of the effect of 3.
Values for $r < \pm 0.20$ noted as 0.

imal value for β fraction (Table 23) However in biliary disease the approximately maximal value for the γ fraction showed a partial, positive correlation with the reduced index for serum bilirubin after elimination of the effect of temperature, and positive correlation with temperature after elimination of the effect of the bilirubin. Finally after elimination of the effect of the γ fraction value a negative correlation was noted between the fever and jaundice in biliary disease.

Approximately simultaneous and maximal values *for serum bilirubin were positively correlated with the β* fraction and the γ fraction in biliary disease, but simultaneous values for serum bilirubin and β fraction were not correlated (Table 24 and Fig 51)

0 Relationship between the serum albumin and other features

In bacterial pneumonia (Table 25 and Fig. 52) the reduced index for serum albumin was negatively correlated with all the primary inflammatory reactants and with the γ and β fractions. In the other diseases studied (Fig. 50) the same negative correlation was found between albumin and body temperature, and in the bacterial infections also between albumin and γ fraction. But in mononucleosis the serum albumin was, if anything positively correlated with the α fractions. A few patients with acute bacterial meningitis studied, all of them severely ill showed a positive correlation between serum albumin and β fraction (Fig 52) In acute bacterial pneumonia no

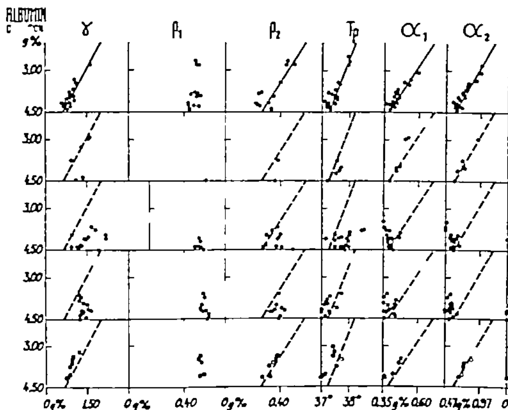


Fig. 32. Relation between the reduced index of serum albumin decrease and the levels of the serum γ-, β- and β fractions and the reduced index of different primary inflammatory reactants in various diseases. The lines of regression for the albumin decrease on the other features in pneumonia is drawn also in the fields of the other diseases.
°) Values obtained from sera which have been kept in the frozen state.

Table 25. *Relation between the reduced index of serum albumin, and the level of serum γ and β-fractions (during 11—30 days of disease) and the reduced indices of body temperature, α-fractions and CRP in bacterial pneumonia.*

Correlation of *albumin* with

γ	β_1	β	Temperature	α_1	α_2°	CRP
— 0.84***	— 0.73**	— 0.39	— 0.69	— 0.83***	— 0.84***	— 0.78***
n 24	n 19	19	21	21	21	21

° — Seen Belfrage

such correlation was found in spite of the fact that the main component of the β fraction, the transferrin, varies with serum albumin in infection.

7 Relationship found on χ^2-analysis and further analysis, between features of infectious mononucleosis and infectious hepatitis

The relationship in infectious mononucleosis Data obtained in 208 cases of mononucleosis in patients aged 9—23 years and representing three fourths of the entire material of mononucleosis were studied by the χ analysis.

In Table 26 14 features are correlated. Four of them showed a tendency to positive correlation with a quotient of at least +2.2 in 5 of 6 possible cases. These features were a_1, a_2, fibrinogen and CRP thus all of them primary inflammatory reactants Another group of 4 features showed a tendency to be positively correlated with a quotient of at least +1.8 in 5 of 6 cases. These were the γ fraction, thymol turbidity value, size of spleen and relative number of mononuclear cells in the blood, thus all of them secondary inflammatory reactants The 4 primary reactants mentioned were negatively correlated with the number of mononuclear cells and if anything also slightly with the other 3 secondary reactants. However the CRP like the maximum number of the polymorphonuclear cells was positively correlated with the γ fraction.

The picture of mononucleosis thus included features which appear to be positively correlated with each other within two groups, one group belonging to the primary inflammatory reaction the other to the secondary reaction. The 2 groups on the other hand showed a tendency to be negatively correlated with one another

The duration of fever and the patient's age were negatively correlated with primary inflammatory reactants, but positively correlated with secondary reactants.

The results of the χ analysis in this mononucleosis series were only partly confirmed by further analysis. When the maximum values noted for the different features were plotted against another a weak correlation was found between some of the primary reactants. But no obvious correlation was demonstrated between the secondary reactants, except between the thymol turbidity value and other components, and, finally no distinct negative correlation was found between the two kinds of reaction, but weak negative correlation was found between age and the a_1 fraction.

On the other hand, a positive time relationship though weak, was found in individual cases between the γ fraction, and the P B titer and the relative number of mononuclear cells.

Of patients with mononucleosis not included in the χ analysis, the *younger group* 29 children *below 9 years* usually had fever of shorter duration and P B reaction of lower titer than the patients between 9 and 23 years. Thirty *patients above 23 years* usually had fever for a longer time. They had lower values for a_1 fraction haptoglobin and fibrinogen, but higher values

Tabl 26 *χ-analysis of values fo vari us features in infectious mononucleosis*

	E m x.	B m v.	CRP m x.	Fibrinogen pl m v.	Polymorphs max./cu mm	Polymorphs min. cu. mm.	Fibrinogen/pl. min.	E. S. R. m x.	γ- E	Thymol t rbidity max.	Length of splee	Mononuclea cell m x. %	Fever d ration	Age
α_1 max.		(101)	(88)	(47)	(88)	(83)	(40)	(102)	(102)	(92)	(64)	(99)	(102)	(117)
α_2/a m x.	+2 6		(65)	(45)	(83)	(81)	(40)	(99)	(95)	(89)	(65)	(97)	(101)	(119)
CRP/ max.	+2 8	+2.8		(45)	(85)	(82)	(38)	(91)	(83)	(83)	(66)	(89)	(90)	(107)
Fibrinogen pl max.	+2.0	+2.2	+1.0		(47)	(44)	(27)	(48)	(44)	(45)	(36)	(47)	(46)	(57)
Polymorphs ma n. mm.	0	+1 6	0	0		(94)	(41)	(97)	(83)	(89)	(66)	(98)	(91)	(116)
Polymorphs min./cu. mm.	+1 4	+1.2	+1.8	+1.6	0		(40)	(93)	(79)	(89)	(66)	(94)	(93)	(112)
Fibrinogen/pl min.	0	+1.3	0	0	0	+2.2		(42)	(39)	(40)	(35)	(43)	(44)	(51)
E. S. R. max.	0	+2.5	+2.0	0	+1.3	0	0		(98)	(188)	(72)	(204)	(202)	(244)
γ/a m x.	−1 4	0	+1.6	−2.1	+2 1	−1 2	−1 1	+2.5		(89)	(64)	(96)	(98)	(119)
Thymol turbidity m x.	−1.8	−1.0	−1 4	0	0	−2 0	0	+1.3	+4 2		(68)	(180)	(188)	(238)
Length of spleen	−1.5	0	0	−1 3	0	0	−2 5	0	+1 5	+2 7		(71)	(72)	(81)
Mononuclea cells max. %	−3 6	−2.8	−2 2	−2.5	−2 0	−2 7	0	0	0	+3 4	+1.8		(208)	(271)
F ver duration	−1 6	0	−1 7	−2 0	0	−1 1	−1 2	0	+1.5	+2 9	+1 8	+2 6		(277)
Age	0	−3 4	0	−2.5	0	0	−1.8	−1.8	+3.2	+1 2	0	0	+2 8	

V lues in the left-lower part of the table Quotient $\frac{O-E}{\frac{\sqrt{N}}{4}}$

Values in brackets Number of observations

P	Value of Quotient
0 05	2.0
0 01	2 6
0 001	3.3

for aldolase, thymol turbidity and γ fraction, and they had more fre quently fibrinolysis.

Fourteen *jaundiced* patients with mononucleosis were on the average older· they had fever for a longer time and they had higher aldolase and thy mol turbidity values. The γ and β

fractions were larger and the values for the CRP α_2, haptoglobin and fibrinogen were somewhat lower than *the corresponding means for the group* analysed by the χ test.

In accordance with the findings in the χ-analysis the score *i.e.* the sum of points for 5 different primary inflammatory reactants was found to be *negatively correlated with the score* of 3 secondary reactants, but rather weakly r = −0.39* (n 38)

Splenomegaly is usually associated with polymorphopenia and a relatively low α_2 value and often with a low fibrinogen value. When the "length of spleen" was excluded from the secondary reactants a significant negative correlation was no longer demonstrable. Neither was any correlation found after exclusion from the series of 5 icteric patients. All of them had a very slight primary inflammatory reaction, but a marked secondary reaction, as judged by the scores.

Consequently the tendency in this material of mononucleosis to a negative co-variation between some components of the primary and secondary inflammatory reactions is only weak. It seems to be in part connected with the degree of "hypersplenism" and jaundice and age

The relationship in infectious hepatitis Of 52 cases of probable infectious hepatitis only 38 patients, aged 8—40 years, were included *i.e.* three fourths of the total material.

Fourteen features are studied in table 2. The table shows a slight tendency to positive interrelation between primary inflammatory reactants α_2, CRP and fibrinogen, on one hand and secondary reactants γ fraction, time of hypergammaglobulinaemia and thymol turbidity value *on the other hand.* Features indicating hepatocellular damage *e.g.* bilirubin value and aldolase value, as well as the albumin level, which may also be included here did not show any distinct correlation with *the primary or secondary reactants.* Only serum bilirubin and CRP was found to be positively correlated.

Thus, *in hepatitis*—in contrast to mononucleosis—there was *no tendency to a negative correlation* between the primary and secondary inflammatory reactions. The primary reaction, however was very weak in hepatitis. Signs of hepatocellular damage *e.g.* serum bilirubin and aldolase, were largely independent of primary and secondary reactants but age and, to a less extent duration of fever were positively correlated with all 3 groups of features.

By means of plotting in a co-ordination system, the positive correlations between age and several other features suggested by the χ analysis were verified, and also the relationship between CRP and the duration of fever and the maximal height of serum bilirubin.

The values for the β fraction and E. S. R. were also found to be correlated.

Analysis of the cases not included in the χ analysis showed that *children* had less CRP lower α_2 fraction and aldolase value and particularly a shorter duration of fever of albumin decrease and thymol turbidity change.

Table 27 *χ-analysis of values for various features in infectious hepatitis*

	γ1/ m	CRP m x	Fibrinogen/pl m x	Polymorph (1st v lu)	E.S.R. max.	γ/ max.	Time of elevated γ	Thymol turbidity m x	Bilirubin max.	Aldolase max.	Albumin's min.	α/ m x	Fever duration	Age
γ1/ max.		(37)	(19)	(28)	(35)	(38)	(35)	(38)	(38)	(24)	(38)	(38)	(38)	(50)
CRP max.	+1.9		17)	(37)	(37)	(35)	(37)	(37)	(37)	(22)	(37)	(37)	(37)	(49)
Fibrinogen pl max.	+1.9	+1.7		(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(49)
Polymorphs (1st value)	0	−1.2	−1.8		(28)	(27)	(28)	(28)	(25)	(24)	(28)	(28)	(28)	(36)
E.S.R. max.	+2.0	0	+2.2	0		35)	(38)	(38)	(38)	(24)	(38)	(38)	(38)	(49)
γ/ max.	+1.9	+2.7	+1.5	0	+1.9		(38)	(38)	(28)	(24)	(38)	(38)	(35)	(50)
Time of elevated γ	+2.3	+2.8	+2.4	0	+1.3	+3.9		(38)	(38)	(24)	(38)	(38)	(35)	(47)
Thymol turbidity m x.	0	0	+1.8	0	+3.2	+2.6	+1.9		(35)	(24)	(35)	(35)	(35)	(51)
Bilirubin max.	0	+2.8	0	−2.0	0	+1.9	0	0		(25)	(38)	(38)	(35)	(51)
Aldolase max.	0	0	+1.9	−1.8	0	0	+1.2	0	0		(24)	(23)	(25)	(33)
Albumin's min	0	−1.8	−1.3	+1.5	−1.6	0	0	−1.0	−1.6	−2.2		(38)	(38)	(50)
α/ max.	+2.0	0	+1.3	−1.2	+3.6	0	0	+2.3	+1.0	0	−1.0		(28)	(51)
Fever duration	0	+3.1	0	−1.9	0	−1.6	0	+1.8	+2.6	0	−1.3	+1.3		(51)
Age	+2.8	+3.8	+4.1	17	+1.0	+1.1	+4.0	+2.7	+1.0	+3.0	−2.1	0	+2.4	

Values in the left lower part of the table: Quotient $\frac{O - E}{\frac{\sqrt{X}}{4}}$

Values in brackets: Number of observations.

P	Value of Quotient
0.05	2.0
0.01	2.6
0.001	3.3

Patients above 40 years had a higher CRP value, aldolase value and thymol turbidity value, as well as a longer duration of fever jaundice, γ increase, albumin decrease, and thymol turbidity change than patients 8—40 years of age

Consequently age seems to be a main determinant of the picture of this disease

Chapter V

GENERAL DISCUSSION

PART I

DEFINITIONS

The inflammatory changes found in this and other materials of infectious diseases can as mentioned in Chapter II be divided into two groups of reaction with different time tables the primary and the secondary inflammatory reactions of ODENTHAL. He included, however only the plasma protein changes in these terms. Here the terms have been used in a broader sense including all sorts of changes connected with inflammation in infectious disease But the conception of the word inflammation divided into two parts according to the time of their appearance is not quite satisfactory In fact this time table holds only for changes in acute bacterial infection. In virus infection such as hepatitis signs of both primary and secondary reactions are present from the beginning of manifest disease, as well as in hyperergic disease such as rheumatic fever In these diseases it is difficult to know what is primary and secondary not only in time but also in the pathogenesis.

In Chapter II it is shown that the primary inflammatory reaction is common to most sorts of injury When according to definition, inflammation is the local response to injury this is particularly the case with the primary inflammatory reaction. MACFARLANE (1962) writes Any damage to the tissues whether it be mechanical, thermal chemical or toxic, will produce some degree of the local reactions included in the term inflammation. It is not so well recognized that such damage may also produce in the plasma and cells of the blood changes which are a part of the general *reaction to injury*

The secondary reaction is concerned with the antibody formation. This is surely a part, although not always evident, of each infectious disease. But in inflammation and necrosis not associated with any exposure to foreign antigen, one would not expect to find antibody formation to be a component In other words, the secondary reaction may not be a regular part of inflammation as is the case with the primary reaction. On the other hand, antibody formation may perhaps occur without signs of a primary inflammatory reaction present.

In the following discussion the terms of ODENTHAL will be replaced by two terms which differ with respect to

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Italicized by present author

pathogenesis. Instead of the primary *inflammatory reaction* MacFarlane's term *reaction to injury* will be used, and instead of the secondary reaction the term *reaction to antigen*

Not every form of injury however I followed by inflammation, for xample not the injury caused by tetanus toxin or other kinds of cytotoxic effect. An in ol e-ment of the mesenchyme seems to be the prerequisit for the occurrence of a reac tion to injury in the sense used here

On the other hand, "Immune reactions are reactions to the injury which is elicited by antigen antibody union. They are not reactions to antigen in the sense used here

The discussion which follows is concerned with the reaction to injury the reaction to antigen and, finally with the relationship between these two aspects of inflammation caused by in fection.

REACTION TO INJURY IN ACUTE INFECTIOUS DISEASES

The reaction to injury comprises most of the symptoms and signs of acute infection, local signs of inflammation as well as fever leucocytosis, elevation of the E. S. R. and increase of the α fractions and fibrinogen.

These components of the general reaction to injury represent only some changes elicited by the injury

Different sorts of injury produce largely the same reaction, probably because they generally affect the most sensitive elements of the tissue first. The first morphological sign of tissue damage, at least in many kinds of acute inflammation, is the loss of granules in the mast cells (ROBB-SMITH 1957) These granules contain heparin and histamine which are thereby released. The further course of the reaction to injury is dictated by different sorts of endogenous mediators rather than by the character of the injurious agent. Thus, the reaction to injury is largely the same whether the damage is due directly to an infection, e g streptococcal infection, or mainly to sensitization as in rheumatic fever

The uniformity of the reaction to injury produced by different agents suggests a close relationship between the various components of the reaction.

In infection the proliferation and elimination of pathogenic organisms result in a course of the changes differing from that in other types of injury This characteristic of infection is however not sufficient to distinguish infections from other types of injury with certainty Only *conditions with a strong but transient reaction to injury in association with a somewhat late increase of the γ fraction are almost invariably bacterial infection or disease secondary to bacterial infection*, such as rheumatic fever

COMPONENTS OF THE REACTION TO INJURY

FEVER

Judged from the induced fever experiments, fever occurs promptly in acute infectious disease—within a few hours—and disappears as soon as the process has regressed (Figs. 35—37) Fever sets in usually as the first sign of acute infection, often together with leucocytosis. In myocardial infarction and after operations fever does not occur until one day after but also in these conditions leucocytosis is evident already some hours after the event (FORSSMAN 1954 SCHUMACHER 1960 Fig 34)

In bacterial infections there appears

to exist a correlation with other components of the reaction to injury (Figs. 38, 47 Tables 15 18) In virus infections the fever was at most weakly correlated with the other components of the reaction and as a rule it was stronger than in a bacterial infection with an otherwise corresponding reaction. This disproportionately high fever in virus infections suggests that the fever inducing mechanism differs from that in bacterial infections. Animal experiments with influenza virus (ATKINS & HUANG 1958) and with Coxsackie virus culture (KING 1962) suggest that in virus infection fever is elicited by an endogenous pyrogen not emanating from the leucocytes.

A febrile reaction that is strong, compared with other components of the reaction to injury is seen not only in virus meningitis but also in bacterial meningitis (Fig. 47) The high fever in meningitis is probably to some extent a direct result of the action of infectious toxins on the brain, in agreement with the findings in experimental animals by BENNETT et al. (1957)

The duration of fever in mononucleosis (Table 26) was found to be positively correlated with the signs of the reaction to antigen, and, if anything, negatively correlated with the other components of the reaction to injury Judging from these findings, the persistence of fever in mononucleosis may depend mainly on the lymphoid reaction, *which is especially* strong in this disease. But more probably the findings are explained by splenomegaly and some other features in mononucleosis with a moderating influence on reactants to injury other than body temperature (see page 132)

LEUCOCYTOSIS

The marked collection and destruction of polymorphonuclear cells in the inflammatory foci seen in infections with exotoxin producing bacterial agents has its counterpart in the leucocytosis in the blood This was the common finding in the present material of febrile bacterial infections.

In tuberculosis, typhoid and paratyphoid fever an increase in the number of polymorphonuclear cells occurs only if pyogenic infection is superadded. It occurs, as a rule, not in virus infections (MACFARLANE 1962) This holds also for the present series. Particularly in infections with associated splenomegaly leucopenia is common. In the present material splenomegaly and a sub-normal or normal number of polymorphonuclear cells during fever were seen in cases of mononucleosis, hepatitis, salmonellosis, subacute bacterial endocarditis and toxoplasmosis. Leucopenia was most pronounced in the aftercourse of mononucleosis, the disease which showed, on the average the largest increase of the spleen (Figs. 22 and 38)

In states of splenomegaly the leucopenia has been ascribed to an increased elimination of the leucocytes in the spleen (WISEMAN & DOAN 1942 JANDL et al. 1961) or to a change in the humoral control of the release from the bone marrow which is generally hyperplastic in these conditions (HEILMEYER 1955)

The relationships in bacterial dis-

eases between the number of polymorphonuclear cells in the blood and the protein components of the reaction to injury were generally weaker than the inter relationships of the latter and often only questionably positive (Table 16 and Fig 41) In the experiments with induced fever the correlation was more evident (Fig. 42)

Also the positive relationship found by ODENTHAL (1958) by means of χ analysis, between the leucocytes and the α-globulin and fibrinogen in acute inflammatory conditions was weak, like the positive relationship found by MÄRKI (1957) between polymorphonuclear cells and α-globulin.

However considering the great difficulties in assessing the relationship between morphological and humoral elements of inflammation, the findings support WUHRMANN's conception of such a relationship These difficulties have received special attention in the present work.

C-REACTIVE PROTEIN (CRP)

CRP cannot be demonstrated in normal serum, but it appears in inflammation or necrosis of some degree, irrespective of its cause. CRP seems to occur only in the presence of necrotic cells. Immunohistochemical investigations (KUSHNER & KAPLAN 1961) suggest that CRP after intramuscular injection of typhoid vaccine is present in necrotic muscle fibres, but not interstitially or in leucocytes.

Of the protein changes observed and assigned to the reaction to injury the appearance of CRP in the serum is the one which occurs first (Figs. 35—37) *Like the degree of fever and leucocytosis its amount reflects the injury independently of its duration.*

The bulk of the CRP appears within about 24 hours of an injury although traces appear already within 6—8 hours after the injection of an endotoxin (Fig. 35) and postoperatively (RAPPORT et al. 1957)

As expected from the work of HEDLUND (1947) every febrile, and also often afebrile, bacterial infection in the present material was accompanied by the appearance of CRP in the serum, which was found correlated with the other components of the reaction to injury (Fig 40) Febrile conditions which are due to cancer ischaemic necrosis, rheumatic fever or rheumatoid arthritis are also regularly associated with CRP in the serum. In some sorts of virus infection, on the other hand, CRP was most often missing also during high fever Judging from personal experience *virus infections are the only common cause of fever without CRP in the serum*, a fact which is sometimes of diagnostic importance. This has received attention by JANSSON et al. (1959) in the differentiation between bacterial and viral meningitis.

The absence of CRP in the serum in many febrile cases of virus infections is a manifestation of the general discrepancy between a marked fever and an otherwise slight reaction to injury in this type of disease. This slight reaction to injury is probably due mainly to the virus acting intracellularly while most bacteria are usually intercellular and act upon the mesenchyme

as a whole In virus infections affecting mucous membranes and skin with necrosis and supervening bacterial infection, the serum contains a definite amount of CRP Thus, about two thirds of the present cases of mononucleosis had CRP in the serum in the acute stage.

In infectious hepatitis, CRP occurred although only in a small amount, even in patients with slight fever and sometimes during the afebrile stage (Fig 40) That CRP often occurs in this disease has been stressed by WOOD & MCCARTY (1951) and HEDLUND (1961) CRP was demonstrated in most of the adults with infectious hepatitis in the present investigation, but hardly ever in children, who as a rule had only mild spells of the disease

HEDLUND (1961) has also found CRP more often in elderly patients with hepatitis than in younger and he demonstrated a correlation between CRP and the duration of the disease as judged from the serum bilirubin and the thymol test. The present series of hepatitis suggests a correlation between CRP on one hand, and fever duration and jaundice, as well as age, on the other (Table 27) It is possible that necrotic liver parenchyma cells are the source of the CRP in the serum in hepatitis. *On the other hand, the* increase of the specific enzymes is a much more sensitive sign of hepatocellular *damage than CRP* When a case of hepatitis showed CRP in the serum, the aldolase activity—or G P T —was regularly pathologically increased, and the patient often showed a moderate increase even when no CRP was demonstrable.

INCREASE IN THE α-GLOBULINS AND FIBRINOGEN

In this material of infectious diseases the reaction to injury nearly always comprised an increase of the α fractions and most often of the fibrinogen and the E. S R value

This increase occurred in the experiments with induced fever as well as in acute bacterial diseases (Figs. 35—3) with a lag of about 12 hours and reached its height the 3rd—5th day after onset. Also after myocardial infarction (LINKO & WARIS 1955) and after operations (SCHUMACHER & SCHLUMBERGER 1962) the increase is slow and culminates during the 3rd—5th day

While as mentioned, fever leucocytosis and amount of CRP in the serum reflects the intensity of the injurious process irrespective of its duration, *the increases of the α fractions the haptoglobin, the fibrinogen and of the E S R. value do not reflect the instantaneous intensity of the process but the sum of effect during a certain period* one or two weeks, preceding the determination. An infection or other injury which is active for only some hours to a few days, therefore *causes only a relatively slight increase* of the α fractions and fibrinogen, even if it is very active as reflected by a high grade fever and leucocytosis and abundant CRP (Figs. 38, 39 and 41) Activity of at least 3—4 days duration is necessary to cause a strong disorder of the above mentioned proteins, and

after the regression of the disease the activity of the injury suffered is still reflected in raised levels of the α fractions and the fibrinogen for 1—2 weeks.

As a rule, the α fraction increases about half as much as the α_2 fraction. The fibrinogen does not usually reach the level of the latter fraction.

Generally *in acute bacterial disease and in atypical pneumonia*, the disorders of the α_1 fraction the α_2 fraction, the haptoglobin and the fibrinogen are well correlated (NYMAN 1959 and Table 17) But the α_2 fraction changes somewhat slower than the α_1 fraction (SCHEURLEN 1955 and Fig. 43) because of constituents other than the haptoglobin with a slower increase and regression (NYMAN 1959 SCHUMACHER & SCHLUMBERGER 1962)

In certain infections this correlation was less distinct or not demonstrable particularly *in infections with marked lymphoid proliferation*. In hepatitis and mononucleosis, the values for haptoglobin and fibrinogen were low and not correlated with one another or with the α_1 fraction (Figs. 44—46) In typhoid fever sarcoidosis and subacute bacterial endocarditis, as in hepatitis, SCHEURLEN (1955) found the quotient α_1/α_2 to be higher than in other infections. Also in other types of salmonellosis in the present material and particularly in mononucleosis the same tendency was seen and also somewhat low haptoglobin and fibrinogen values in relation to the α_1 fraction (Figs. 43—45)

Infections in this series with lymphoid proliferation very often had *splenomegaly* This may be responsible for the relatively low values found for the α_2 fraction, the haptoglobin and the fibrinogen. JANDL (1961) claims that splenomegaly in infectious conditions is related to the prolonged fever and the concurrent stimulation of the R.E.S., and that the splenomegaly in its turn results in increased haemolysis by increased sequestration of the red blood cells. This haemolysis is usually reflected by a relatively low haptoglobin and α value. Repeated administration of endotoxin in animals was also followed by an activation of the R. E. S and resulted in splenomegaly and in anaemia due to increased haemolysis (HO & KASS 1958)

The relatively low fibrinogen content of the plasma, particularly in mononucleosis, was due at least partly to an increased fibrinolysis Dr I M NILSSON found fibrinolysis in many of the cases of mononucleosis and hepatitis in this series. NYMAN (1959) found in mononucleosis a negative correlation between the degree of fibrinolysis and the haptoglobin level.

Fibrinolysis in the present material was, however most regular in the experiments with endotoxin during the first phase of its action, together with a low fibrinogen value and sometimes leucopenia and a slight decrease of the haptoglobin.

Probably low values for haptoglobin and fibrinogen are in some way connected features, also related to the lymphoid proliferation in these diseases and particularly to the splenomegaly They are probably most often

due to increased elimination This applies, as mentioned earlier also to the leucopenia. In hepatitis and other kinds of injury to the liver decreased synthesis may be partly responsible

A further feature common to hepatitis, mononucleosis and toxoplasmosis in this material was the relatively high total protein value in the later course of the disease (Figs. 17—22 and 31 32) HAVENS (1946) followed the total protein at close intervals in 29 patients with inoculated infectious hepatitis and found an increase above the normal mean from the 10th day of disease during the entire observation period of three months

After the first month of disease the serum albumin was usually normal in HAVENS and in the present series. Evidently the increase of the total protein cannot be explained by any mechanism for the maintenance of the colloidosmotic pressure. Perhaps it reflects an abnormally large extravasation in the portal region of protein-poor fluid, and such a disorder may have a connection with other changes in the aftercourse of these diseases.

Whether the reaction to injury *in toto* is less marked after a *repeated or "chronic" injury* than after a single injury cannot be deduced from clinical experience. In animal experiments SCHEURLEN (1955) assessed the serum protein changes after injections of casein lye in mice prepared and not prepared respectively by injection of the *same irritant some weeks before*. He found a weaker α increase but a prompt γ increase in the pre-treated animals. Here immunity to casein may have been the deciding factor Similar experiments with a non antigenic injurious substance are not known to the author

In clinical work only discrepancies between the components of the reaction can be recognized. Supervening injury by endotoxin, in the chronic infected state resulted in fever leucocytosis and increase of the CRP and also of the protein bound hexoses in the serum but without any distinct change of the α fractions, haptoglobin and fibrinogen (PROBST et al. 1958 and the present experiments with endotoxin, Fig 35 c)

In *biliary disease* with fever sometimes no leucocytosis was noted and, compared with cases of pneumonia with the same degree of fever the increase in both α fractions was somewhat low The negative correlation found between fever and jaundice in biliary disease although uncertain, may also be mentioned These findings may indicate a weaker reaction to injury in biliary disease with jaundice than in other bacterial disease in agreement with findings in the experiments of SELYE et al. (1954) namely a slighter inflammation in the form of exudation in animals with mechanical jaundice than in anicteric controls.

In the *bacterial pneumonia* series the fibrinogen values were sometimes found to be low relative to the α_1 values (Fig. 45) This is in accord with SCHULZ's finding (1953) of a relatively low fibrinogen in bronchopneumonia, particularly in elderly people. Subnormal values in the later course of the

disease as reported by SCHULZ to occur in elderly patients were not seen in the present material.

Another type of discrepancy between the two α fractions was recorded in *virus meningitis* In the febrile stage cases of this group most often showed a normal or nearly normal α_1 value with a moderately increased α_2 fraction (Figs 38 43 and 48) while patients with acute bacterial meningitis had an increase of the α_1 fraction in proportion to the increase of the α_2 fraction (Fig 48) In the patients with virus meningitis CRP in the serum was also missing

The absence of an obvious increase of the α_1 fraction in virus meningitis may be due to the low activity of the inflammatory process or rather to the scarcity of mesenchymal tissue in the central nervous system.

MECHANISM OF THE REACTION TO INJURY

The uniformity of the reaction to injury in association with different sorts of damage can probably be explained by the assumption that certain mediators are regularly thrown into activity It is known that in some conditions endogenous pyrogen is a promoter of fever and that other endogenous mediators—not active in virus infection—promote leucocytosis.

The mechanism responsible for the increase of the α glucoprotein reactants to injury and of fibrinogen is not properly understood. The lag phase and the dependence of the duration of the injury suggest that these protein disorders are not as direct a link in the reaction to injury as fever leucocytosis and formation of CRP

The close relationship between the reactants to injury in different sorts of infection makes it probable that the capacity of the site of production is, as a rule, sufficient—for α proteins and fibrinogen this site seems to be the liver—and that the rate of elimination of the reactants varies less than their production. In liver disease and in chronic disease (JARNUM & LASSEN 1961) however decreased capacity of synthesis of the above-mentioned and other proteins may limit their amount in the plasma. In infection with splenomegaly the low values found for haptoglobin and fibrinogen as well as for polymorphonuclear cells may be due to increased elimination or possibly to a low production.

REACTION TO ANTIGEN IN ACUTE INFECTIOUS DISEASES

While the clinical picture of an acute infection is dominated by the reaction to injury the reaction to antigen is usually not clinically manifest until its final result—immunity or sensitization—modifies or manifests the picture of the disease. The chain of events between the exposure to antigen and the above-mentioned result usually gives only indistinct clinical signs. Only in a small number of infections does the increased activity of the lymphoid apparatus produce a clinically demonstrable enlargement of the *spleen and lymph nodes or distinct* changes in the bone marrow and blood cells, although small lymphoid changes difficult to assess must occur in all cases. The γ-globulin content of the serum increases in many infections, but in most of the acute infectious diseases the γ-fraction is normal. Specific antibodies arise in various infections, but they are clinically demonstrable in only a part of them

ENLARGEMENT OF THE ANTIBODY FORMING ORGANS AND CELLULAR SYSTEM

Substantial enlargement of lymphoid organs was seen in certain infections. The organs affected were mainly the spleen and the lymph nodes. This swelling of the lymphoid organs was, as a rule accompanied by a mononuclear blood cell picture and by an increase of the γ fraction in the serum

Most of the cells of lymphoid tissue take part in antibody formation. ROBERTS and GOWANS (1962) write An tibody formation takes place where R. E. elements are associated with lymphocytes plasma cells and primitive mesenchymal cells Proliferation of these types of cells—diffuse or in the form of granulomata—is most often a manifestation of a reaction to *antigen.*

The mononuclear cells, *i.e* lymphocytes and monocytes, in the circulating blood are potential components of the reaction to antigen In most infections the mononuclear cells of the blood were normal or increased relatively slightly in number This slight increase, especially in the later course of bacterial disease, corresponds to SCHILLING's monocytic Kampf phase and lymphocytic Heil phase (SCHILLING 1912) In virus infections only relative lymphocytosis was usually found. The immunizing activity is reflected perhaps more often in a qualitative change of the mononuclear cell picture of the blood than in a quan

titative change. Lymphatische Reaktionsformen are seen in different kinds of infection (KLIMA 1961) KLIMA includes in these the large lymphocytic cells with basophil cytoplasm, seen above all in mononucleosis, but also in many types of virus infection (LITWIN & LEIBOWITZ 1951) in protracted bacterial infection and in toxoplasmosis. The basophilia of the cytoplasm is due to an increased content of ribonucleic acid, the presence of which is a sign of increased protein synthetic activity (CASPERSSON & THORELL 1941) Also the plasma cells, which are seen in the peripheral blood above all in rubella, are regarded by KLIMA as belonging to the "lymphatische Reaktionsformen

Fig 5 shows a case of very severe pneumonia with marked cytologic signs, in the bone marrow and in the blood of a reaction to injury and a reaction to antigen—the latter in the form of an increased number of plasma cells and, as humoral counterpart, a marked increase of the serum γ globulin.

The 15 cases of pneumonia—some of short duration—in which the bone marrow was studied showed signs of both myeloid proliferation and plasma cell increase without any distinct correlation with the level of the serum γ globulin (Table 7)

GORMSEN (1942) also found an increase in the number of plasma cells in the bone marrow in different sorts of infections with marked increase of the serum globulin such as typhoid, sepsis,and (1948) chronic hepatitis. On the other hand, JESCHAL (1953) found no increase in scarlet fever diphtheria and tonsillitis.

A positive correlation between the number of bone marrow plasma cells and the serum γ fraction was found by JARROLD & VILTER (1949) in cirrhosis but not in hepatitis, and by GOOD & CAMPBELL (1950) in rheumatic fever

A correlation between the serum γ globulin and the morphological elements of the blood has been reported mainly in infections with an obvious lymphoid reaction. In mononucleosis BEYREDER & RETTENBACHER DÄUBNER (1953) found a positive correlation between the γ fraction and the lymphatic blood cell picture. In the same disease in the present material a time relationship but no distinct quantitative relationship was found between the γ fraction, the Paul Bunnell titer and the number of mononuclear cells in the blood, and a rather weak positive relationship between some other features concerned in the reaction to antigen, as indicated by the χ analysis In hepatitis WUHRMANN and MÄRKI (1960) found a time relationship between the γ fraction and the number of monocytes in the blood.

On the whole, the reaction to antigen is reflected by the mononuclear cells in the blood only weakly in most infectious diseases and by a distinct increase of the bone marrow plasma cells only in more severe and protracted conditions

CHANGES IN THE γ-GLOBULIN AND ANTIBODY PROTEIN

The greatest advance made possible by TISELIUS electrophoretic method was the identification of the γ globulin in serum and the subsequent discovery that this globulin represents the bulk of the antibody protein (TISELIUS & KABAT 1939) While the albumin molecule is a relatively uniform body the γ globulin molecule is chemically heterogenous. In animals hyper immunized with pneumococcic antigen, as much as 85 % of the γ globulin was found to be specific antibody protein (ASKONAS et al 1960) *Groups* of patients show a positive correlation to a certain extent between the mean level of the γ fraction and the antibody titer (MCCARTY 1954 and the present investigation Figs. 9 and 22) In the *individual* infected patient however no regular correlation between the γ globulin level and the antibody titer has been found. It may be due to the fact that the antibody titer is only a measure of a minor part of the specific antibody protein, *e g* in WIDAL s reaction only of the agglutinins A part of the new formed γ-globulin may be directed not against the antigen administered but against other antigen —anamnestic response—or it may be inert

In infection the amount of the γ fraction is an express on of the total act vity of the lymphoid system rather than of the degree of immunity against the actual infection, wh ch may on the other hand, be reflected by the antibody titer The level of the γ fraction is, on the whole, an expression of the capacity of the organism to respond to antigen, of the amount of previous exposure to antigen and of the nature and of the intensity and length of the actual infection. To a certain extent it reflects the *chronicity* of the antigenic contact. The relatively slow response to antigenic stimulation and the low turn over rate of the γ globulin with a half life time of about 20 days implies that the γ fraction level found in the serum reflects, above all, the activity of the lymphoid system some weeks previously

A *normal γ-globulin* value was the most common finding in the present material of acute infections and was the rule both in bacterial diseases of less than one week s duration and in virus infections with signs from the resp ratory or digestive tracts or the meninges—most of the virus infections. WUHRMANN's statement that the γ fraction is increased in most virus infections could not be confirmed by the present investigation in which a regular increase of the γ fraction was found only in hepatitis and mononucleosis

Hypogammaglobulinaemia of moderate degree was noted in several patients. Some showed a tendency to repeated nfections, others did not The low γ globulin was probably most often a manifestation of a low capacity to form antibody or in children of a sl ght prior exposure to antigen

In cases of dermatitis and enteritis with low γ and total protein values the decrease was probably due to loss of protein from the blood stream

Hypergammaglobulinaemia was the rule in the more severe cases of bacterial infection with fever of more than one week's duration, in diseases secondary to bacterial infection of the type rheumatic fever and in virus infections of the type hepatitis.

The increase of the γ fraction in *acute bacterial disease* was positively correlated with the intensity of the previous reaction to injury. It was a link in a general disorder of the protein pattern, and it disappeared at most some weeks after the other protein disorders (Fig. 4 b).

In *hepatitis and mononucleosis* the increase of the γ fraction, both absolute and relative to the intensity of the reaction to injury, was large and not correlated with this reaction (Figs. 17—22). It occurred as a predominant disorder of the electrophoretic pattern and persisted often a very long time, denoting further activity of a pathological process. The increase of the γ fraction was manifested by an elevated value for the thymol turbidity in hepatitis and mononucleosis, but usually not in bacterial infections.

The special character of the rise of the γ fraction in hepatitis and mononucleosis may be due to an infection of the cells of the lymphoid system by the virus (WALDENSTRÖM et al. 1951).

Agents, such as Salmonella, Brucella, Leishmania and Toxoplasma have been shown to proliferate in lymphoid cells. Intracellular infection also by such agents may imply a particular stimulation of lymphoid tissue to proliferate and to produce antibodies (WALDENSTRÖM 1952).

Biliary disease with jaundice represents another special group. In animal experiments ARONSEN (1961) showed that a slight to moderate increase of the γ fraction occurs after 2 weeks jaundice, produced by ligation of the common bile duct. Clinical reports on the γ fraction in biliary tract diseases such as those by POPPER et al. (1951), BRANTE (1952), VIOLLIER (1957) usually describe no increase or a slight increase of the γ fraction in afebrile cases of gall stone with jaundice, and a somewhat larger increase in cholecystitis with jaundice, but generally a marked increase in hepatitis, a difference between obstructive and parenchymatous jaundice of only limited diagnostical value.

The present material of gall stone complicated by jaundice and/or fever showed a somewhat high γ fraction compared with that found in cases of bacterial pneumonia with a corresponding fever and α_2 rise (Fig. 49 b and Table 21). In gall stone both fever and jaundice, when expressed by their reduced indices, showed a positive, partial correlation with the γ fraction (Table 23). Also simultaneously noted values of serum γ and bilirubin showed a positive correlation (Table 24 and Fig. 51). This correlation between the γ fraction and jaundice is in agreement with ARONSEN's findings and is perhaps the expression of an enhanced antibody production in obstructive jaundice.

ARONSEN found this condition to be associated with a considerable change of the circulation through the liver with shunting of blood from the portal vein directly to

the liver veins. Both the parenchymal part of the liver and the R. E. S. part are thus partly shut off. It is possible that in obstructive jaundice antigenic substances from the inflammatory focus, or products which may stimulate antibody formation, as nucleic acid derivatives, are destroyed to a less extent by the R. E. S. part of the liver and are released in larger amount into the circulation and to the lymphoid tissue. In the present material a high γ-fraction value in biliary disease seemed to be due usually to severe inflammation of the gall bladder, less frequently to liver cirrhosis.

In diseases secondary to streptococcal infection, e.g. rheumatic fever—in contrast to what has been reported by other authors—the γ fraction was nearly always found to be increased in the present material (Figs. 6 and 9). This finding, however, fits in well with the assumption that prolonged streptococcal infection, as a rule, had existed before the onset of the secondary disease.

Also in tonsillitis the average γ level was somewhat high compared with that in pneumonia with a corresponding reaction to injury (Fig. 49). OBERMAN et al. (1958) found a strikingly high γ value with a maximum already during the first week of the disease in tonsillitis with swollen lymph nodes in children. OBERMAN believes that every infection markedly involving the lymphoid tissue results in a particularly marked increase of the γ fraction. An alternative explanation is, that different infectious agents induce γ globulin formation of different degrees. RINGERTZ & ADAMSON (1950) found, in rabbits, that streptococcal antigen caused a more marked new formation of plasma cells in the lymph nodes than staphylococcal antigen. This difference in effect between streptococci and staphylococci was confirmed in a human autopsy series.

CHANGES IN THE β_2-FRACTION

Little is known of the behaviour in infections of the two β-fractions separated by LAURELL et al.

In the present material a positive correlation was found between the β and the γ fraction in all kinds of infections studied and between β fraction and different components of the reaction to injury in bacterial pneumonia (Fig. 50 and Table 22).

In acute bacterial infection the increase of the β_2 fraction occurred generally some days before the increase of the γ fraction and often in association with a somewhat lower degree of reaction to injury (Figs. 3, 4 and 6). Many cases of bacterial infection with a normal γ fraction thus showed an increase in the β fraction (Table 13). But usually an increase of both fractions in pneumonia required fever of 38 C for at least one week. In virus infections the β fraction increased, as a rule, only when the γ fraction was *increased, and not regularly even then* (Table 13). The increase of the β fraction in infectious disease is due to an increase of the antibody content of the β zone and, above all, to an increase of the β_1c-globulin, which is the main constituent of the β-band and closely linked to the complement system (see page 13).

BOGDANIKOWA (1961) published a

large series of cases of hyper β globulinaemia, studied with LAURELL's method. Since most of her cases were chronic with marked immunization and with a lymphocytic blood picture she concluded that the increase of the β was a manifestation of antibody formation. She found, however no correlation with the γ fraction.

The small series of cardiac infarction in the present investigation showed an increased β fraction with a maximum in the second to the third week, in spite of the fact that sera were examined that had been kept frozen for several weeks with consequent decomposition of a part of β_{1C} (Figs. 33 and 50) The finding agrees with BOGDANIKOWA's observation of increased β in several cases of myocardial infarction. She believes the finding to be a manifestation of increased antibody content of the β_2, but an increase of β c appears a more likely cause

A relatively low value was sometimes found in the present series for the β fraction relative to the marked increase of the γ fraction in cases of hepatitis, mononucleosis, and sometimes in other kinds of immune disease e.g acute glomerulonephritis (Table 12 and Fig. 6) This may be explained by consumption of β c in association with antigen antibody reactions.

The especially high level of the β fraction in biliary disease and the positive correlation found between simultaneously noted values for this fraction and the serum bilirubin is probably caused mainly by an increase of both β-lipoproteins and the β c in this conditions.

CHANGES IN THE β_1-FRACTION

This fraction showed no correlation in pneumonia with the γ fraction or with the reactants to injury An increase of β antibody and perhaps of β_{1A} globulin in infection produced no regular increase of the fraction as a whole This is because the main component of the fraction, the transferrin, decreases in the same conditions But the very slight elevation of the β fraction found in the aftercourse of severe bacterial infection may reflect such an increase in the immuno globulins (Fig 3) In diseases affecting the liver the transferrin is often augmented with resulting conspicuous increase in the β fraction (Table 12)

PART IV

RELATION BETWEEN REACTION TO INJURY AND REACTION TO ANTIGEN

1 DOES AN INJURY ELICIT A REACTION TO ANTIGEN?

Anamnestic response It is known, that an injury associated with slight exposure to foreign antigen, such as a trivial infection or induction of fever can elicit a reaction to antigen in the form of an anamnestic response *i.e* a re-stimulation of antibody formation without evident re-exposure to the specific antigen. Whether an action not associated with any exposure to foreign antigen is sufficient to precipitate an anamnestic response, is not known. BIELING (1918) produced fever by intravenous injection of "Zimtsaures Natrium into experimental animals that had been vaccinated previously with typhoid and Shiga Kruse, but the injection was not followed by any increase of the agglutinin titer against the above-mentioned organisms. RICHTER (1952) found subcutaneous injection of turpentine which is not an antigen either to produce large abscesses in experimental animals, but no increase in the γ globulin in the following course.

Malignant tumours. The development of tumours does not imply any administration of antigen from without provided that infection does not supervene but the malignant cells and their products may be atypical enough to be conceived by the body as foreign. In many cases of malignant tumour the α_2 fraction is increased and the albumin decreased in association with a normal γ fraction other cases, however show a distinct increase of the globulins despite the absence of any infection

In some kinds of renal carcinoma there is often fever a marked increase of the α fractions and of the fibrinogen. BÖTTIGER (1960) found, on the average a slightly elevated γ globulin level in patients with such tumours without any significant difference from the level of the γ fraction in normals.

Necrosis Myocardial infarction whose pathogenetic background is an ischaemic necrosis, often shows distinct signs of a reaction to injury but no increase of the γ fraction (WITAS 1955 LINKO et al. 1956) No significant increase of the γ-globulin was found after myocardial infarction in the present material (Figs. 33 and 34) A weak correlation between the reaction to injury and the level of the γ fraction in this material of myocardial infarction could, however not be ex

cluded, but it may be ascribable to undetected infection in some of the cases.

From the experience with anamnestic reaction, malignant tumour and myocardial infarction it is not possible to give any definite answer to the question whether an injury or effect not associated with the appearance of exogenous antigen can precipitate a reaction to antigen. However *as a rule this is not the case.*

2. DOES EXPOSURE TO ANTIGEN MEAN AN INJURY?

In other words, is a reaction to injury an obligatory consequence of administration of antigen? Experiments with the administration of antigen in the form of killed bacteria and bacterial products of different types have produced changes arguing somewhat for this possibility. RINGERTZ & ADAMSON (1950) showed in association with subcutaneous administration of such antigen, that a brief phase of polymorphonuclear cellular infiltration was the first change noted in the lymph nodes. MARSHALL (1956) found in the spleen after intravenous injection of killed bacteria a polymorphocellular infiltration and a simultaneous decrease of the lymphocytes as the first component of the cellular basis of antibody production. The polymorphocellular infiltration in these two kinds of experiments was probably a manifestation of injury due to toxins in the bacterial suspension which need not have been identical with the antigen substances.

WARD et al. (1959) studied the histological changes occurring in the spleen of the rabbits after a single intravenous injection of purified bovine γ globulin. They found no signs corresponding to a reaction to injury. Not until the 8th day after the administration of antigen did the first changes occur in the spleen in the form of a lymphoid proliferation.

Judging from this last experiment *a reaction to injury is not an obligatory consequence of an exposure to antigen.*

3. RELATION BETWEEN THE REACTIONS TO INJURY AND ANTIGEN IN ANIMALS

In rabbit experiments with re-immunisation SPEIRS (1958) found a close positive correlation between the number of granulocytes, particularly eosinophilic cells, which after administration of antigen were accumulated locally, and the somewhat later occurring increase in the antibody titer.

If antigen and an adjuvant, *e.g. Freund's adjuvant*, are given simultaneously, the local reaction is more intense and prolonged as well as the antibody formation (FREUND 1947, RAMON 1957). The use of FREUND's adjuvant is necessary for the experimental production of autoimmunity (TERPLAN et al. 1960). It is not properly understood how the adjuvant acts. BURNET (1959) believes the main effect of the adjuvant to imply that the antigen is accessible at a more or less steady rate and for a longer time to the lymphoid cells. Others (RAMON 1957, DALE 1960) believe that a local inflammation caused by the adjuvant

also implies an activation of the mesenchyme with consequent stronger antibody formation.

WOOD (1953) found that the antibody formation in rabbits after administration of antigen in adjuvant was correlated not only with local signs but also with general signs of a reaction to injury. Only those animals which responded to antigen adjuvant with a distinct local reaction as well as appearance of CxRP[1] in the serum showed a marked rise of the antibody titer in the serum.

JOHNSON et al. (1956) showed that *endotoxin* administered simultaneously with antigen or a few days afterwards enhanced antibody formation. Endotoxin preconditions the organism so as to allow acute production of antibody (STEVENS & MCKENNA 1957). The endotoxin affects the first stage of antibody formation, the same stage in which cortisone (BERGLUND 1956) and x-ray radiation (TALIAFERRO & TALIAFERRO 1951) have an inhibitory effect. Endotoxin eliminates most of this inhibition. But the endotoxin has this effect only when it produces a general response of the organism with fever. Tolerant animals show no potentiation. A reaction to injury is evidently a prerequisite for the potentiating effect.

The mechanism of this effect is obscure. The cellular destruction caused by the injury may release nucleic acid derivatives with a stimulating effect on antibody formation (LUECKE & SIBAL 1962). Or the reaction caused by endotoxin, which is thought to be in part an immune reaction (GILBERT & BRAUDE 196) may in its turn confer a general activation of the lymphoid system.

Histological examinations (WARD et al. 1959) of the spleen and other organs after antigen-endotoxin administration showed the same relatively slight reaction to the endotoxin as in the control animals during the first few days after the injection and then essentially the same type of reaction to antigen as in the control animals which received antigen alone (see above), but a reaction which occurred much sooner and which was much stronger.

RICHTER (1952) found that simultaneous injection into rabbits of *ribonucleic acid* and antigen in the form of horse serum, markedly increased the following rise of the γ fraction, but that it did not increase the titer of agglutinating antibodies. TALIAFERRO & JAROSLOW (1960) found nucleic acid derivatives to have a restoring effect on antibody formation in x-rayed animals.

These different animal experiments thus suggest *that at least some sorts of injury affecting the organism in an early stage of antibody formation result in intensification of antibody formation.* This effect of adjuvant and endotoxin probably implies a combined action of injury and antigen.

SPEIR's experiments suggest a positive correlation between the activity of the polymorphonuclear cells in the early stage and the amount of antibodies formed. Whether the possibly

[1] Substance in rabbit corresponding to CRP in humans.

potentiating effect of ribonucleic acid is dependent on its being utilized for template synthesis or in other way is not known

4 RELATION BETWEEN THE REACTIONS TO INJURY AND ANTIGEN IN HUMANS

Clinical experience with virus infections argues against a positive correlation between the reaction to injury and the reaction to antigen In hepatitis and mononucleosis, as mentioned the increase in the γ fraction is usually found early in the manifest disease and is relatively marked, but the signs of reaction to injury are usually slight.

In bacterial diseases, on the other hand, the early reaction to injury may have a part in a reaction to antigen which manifests itself one or more weeks later

Bacterial disease, when protracted and recurrent, gives rise to an increase of the fraction (MALMROS & BLIX 1946) and to a marked increase of the antibody titer (WINBLAD et al. 1947) But patients with acute streptococcal disease which were treated with penicillin at an early stage and thus with a brief reaction to injury usually had no increase of the γ fraction or of the antistreptolysin O titer (ANDERSON and co-workers 1948 KILBOURNE & LOGE 1948 and the present investigation Figs. 6—9)

In 14 children with streptococcal infection, which developed into rheumatic fever LIBRETTI et al. (1956) found a positive correlation between the amount of CRP in the serum during the phase of infection and the antistreptolysin O titer during the subsequent rheumatic phase Thus, a correlation between manifestations of the reaction to injury and to antigen in analogy with WOOD's findings in the above mentioned rabbit experiments.

This general experience is confirmed by the findings made in the present investigation of a positive correlation in acute bacterial infection between reaction to injury on one hand and the level of γ globulins and β globulins at the time of their maximum on the other (Figs 49 and 50)

This positive correlation appears to apply to all types of bacterial infection, also tuberculosis and diseases secondary to streptococcal infection, and to all the components of the reaction to injury (Tables 20—23) The number of polymorphonuclear cells however showed only an uncertain positive correlation.

No such a quantitative relationship between the intensity and duration of the reaction to injury and the subsequent level of immuno-globulin has been observed before

5. BACKGROUND TO THE POSITIVE RELATION BETWEEN THE REACTION TO INJURY AND THE INCREASE OF THE γ-FRACTION IN INFECTIOUS DISEASES

In tuberculosis and diseases secondary to streptococcal infection, the reaction to injury may be due to sensitization which in turn is reflected by the increase of the γ fraction In acute bacterial disease with generally a short incubation time as in bacterial pneumonia tonsillitis and meningitis, sensitization is not the main responsible

factor but the positive correlation between the reaction to injury and the γ fraction is probably of some other origin.

The infectious process may cause two independent reactions, the reaction to injury and the increase of the γ fraction, which co vary because both are manifestations of the infectious process. The absence of any correlation in the virus infections examined in the present investigation may strengthen the probability of this explanation.

A reaction to injury may represent the earliest link in the antibody formation process in agreement with MARSHALL's conception of the polymorphocellular infiltration in the spleen and SPEIR's opinion of the eosinophilic infiltration at the site of antigen injection, both in the very first stage after the administration of antigen. This view is supported by experiments by PAGE & GOOD (1958) who found the presence in the inflammatory focus of a certain amount of neutrophils to be a prerequisite for the following steps in the inflammation with infiltration by macrophages and lymphocytes. FISHMAN (1961) found that the phagocytosis of the antigen by macrophages and its disintegration in these cells was a necessary condition for the following antibody formation in lymphoid cells emanating from lymph gland tissue. One might imagine that in other cases phagocytosis of the antigen by the polymorphonuclear cells and its destruction in them *is the first stage of* antibody formation.

The third and most likely explanation for the correlation between the γ fraction and *reaction to injury is* that the latter is *not a necessary link in the formation of antibodies but a stimulant of such formation in the same way as an endotoxin and an adjuvant.* The mechanisms assumably responsible for their effect may also be active when the reaction to injury potentiates the and antibody formation. Particularly the effect of endotoxin appears to be analogous because potentiation is only obvious in animals which really develop fever and other signs of injury.

If a true correlation exists between the fraction and the reaction to injury one wonders why this does not also occur in virus infections. In hepatitis and mononucleosis the disproportionately intense γ formation is probably explainable as mentioned, by an infection of the actual lymphoid system. In virus meningitis and influenza the reaction to injury is usually so slight that no increase in the fraction should occur if the situation is the same as that in acute bacterial infections.

In mononucleosis even a negative correlati n between the two eactions was suggested by the χ-analysis, but ly partly erified by th further analysis. It is probabl due to an opposite influence on components of the two reactions by several f ctors, such as "hypersplenism and jaundice (or the processes ca sing jaundice in mononucleosis) and, finally age.

A negative correlation between the reactions to injury and antigen was n t demonstrable in an other kind f infectious disease in this material.

PART V

BACKGROUND TO THE VARIOUS PATTERNS OF REACTION IN ACUTE INFECTIOUS DISEASES

Chapter V (page 98) summarizes the patterns of reaction of 4 groups of infectious disease. In Fig 53 the same patterns are given and also for comparison the pattern of myocardial infarction

The previous discussion has given some hints on the background of the patterns. They are on the whole presumably the result of common factors more or less at work in various groups of acute infections.

Injury is the most important factor It generally affects the mesenchyme more severely in bacterial infections than in virus infections.

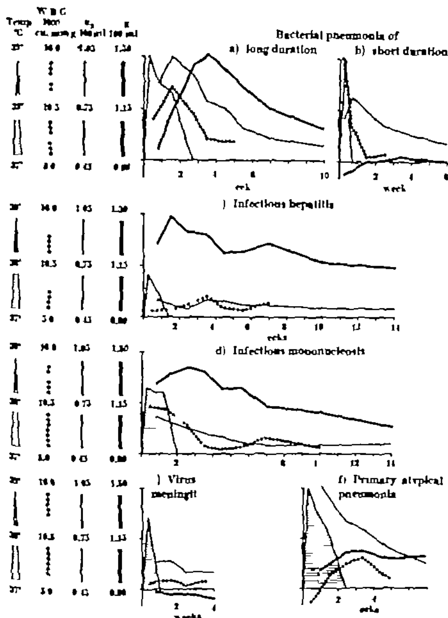
Foreign antigen of the infecting agent causes a distinct rise of the γ fraction only in infection with evident lymphoid proliferation and in bacterial disease only after a relatively severe and prolonged course

A third factor is *time* A few days of manifest disease are necessary to give a distinct increase of the α fractions but more than one week to produce a distinct rise of the γ fraction and less only in some diseases with a long incubation time.

Age influences the plasma protein pattern even in normals Signs of infectious disease, including protein changes, are usually more severe and protracted in older than in younger individuals.

Jaundice in infectious diseases is possibly associated with a slightly modified reaction to injury and a stronger reaction to antigen.

Splenomegaly per se a component of the reaction to antigen, leads to a modification of the reaction to injury probably by means of an increased destruction of leucocytes and erythrocytes



53 and b Pattern in the course of bacterial pneumonia of long duration (a) and short (b) In the former a distinct rise f all factors, in the latter no rise of the fraction.

53 c Pattern i the course of infectious hepatitis. Increase mainly of the γ-fraction.

53 d. P ttern in the course f infectious mononucleosis. Increase of the γ-fraction relatively marked, increase of α_2-fraction and W.B.C.—the latter due t increase of mononuclear cells—relatively weak.

53 e Pattern in the course f irus meningitis Only body temperature distinctly increased.

53 f Pattern in the course of primary typical pneumonia. Distinct increase of the temperatur and α_2 fraction, slight increase f the γ-fraction and in the later course of the W.B.C.

53 g Pattern in the course of myocardial infarction. Increase of temperature, W.B.C. and α_2-fraction, but only slight increase of the γ fraction.

Fig. 53. Reactio patterns in the course of cute infections nd myocardial infarction — mean curves per changes in bod temperature, W.B.C α_2 and γ-fractions

and sometimes a slight increase of the γ fraction—mucosal necrosis and supervening bacterial infection belong to their picture.

The *analysis* of the quantitative relationships did not give accurate results, but there is a great probability that the positive correlations found are true.

Regarding the *time relationship* between different changes, fever and polymorphonuclear leucocytosis developed and regressed promptly with corresponding changes in the activity of the injury as judged from the experiments with induced fever The CRP changed in a corresponding way but with a lag of about one day The α proteins and the fibrinogen reacted slower with a longer lag particularly during the regress of the disease.

The components of the *reaction to injury* showed largely a close interrelationship in bacterial disease but generally no relationship in virus infection—particularly the fever was often disproportionately severe and some other components disproportionately weak in virus disease.

Some components of the *reaction to antigen* as the γ and β fraction, showed a positive interrelationship while the correlation between the γ fraction and the titer of specific antibody and the morphological features was weak or absent. Finally a quantitative correlation was found in bacterial disease between the reaction to injury and the somewhat later culminating γ fraction and, in bacterial pneumonia, also the β fraction. Virus infections, on the other hand, showed no such correlation and cardiac infarction only an equivocal one. In biliary disease due to gall stone the γ fraction was positively correlated with fever and with icterus particularly after elimination of the effect of the other component. Also the β fraction was positively correlated to jaundice

The changes in the β_1 fraction showed no relationship with either of the two reactions.

The albumin level was found to change inversely with body temperature in all types of diseases studied In bacterial pneumonia it showed a negative correlation with the α fractions as well as the γ and β fractions

General discussion The uniformity of the *reaction to injury* produced by different agents implies the evidence that all sorts of injury give rise to on the whole one and the same process, which is mediated largely by endogenous factors deviations in some infections from the general pattern are due to particular mechanisms. Fever leucocytosis and occurrence of CRP in the serum seem to be a more direct link in the reaction to injury than the increase of the α glucoproteins and the fibrinogen as judged from the longer lag of the latter and from their dependence on the duration of the injury

Although infection always implies a *reaction* of lymphoid tissue *to the foreign protein* reflected for example in an increase of the bone marrow plasma cells in pneumonia, the γ fraction forming the bulk of the antibody protein was regularly increased only

in severe long standing bacterial disease and in some sorts of virus infection such as hepatitis and mononucleosis. The distinct proliferation of lymphoid tissue and increase of the γ fraction in hepatitis and mononucleosis, also often in salmonellosis and toxoplasmosis, may be due to infection of the lymphoid cells themselves.

An increase of the β fraction is a part of the reaction to antigen because of its content of antibody protein and particularly of β_{1C} globulin which is closely linked to the complement system. The increase of the β fraction occurred somewhat earlier in the course of an acute bacterial infection and somewhat more frequent than the increase of the γ fraction which generally did not begin until after one week's fever

The mechanisms of the positive relationship found in bacterial infection between the reactions to injury and to foreign antigen are discussed. Evidently as a rule, an injury *per se* not involving any exposition to foreign antigen does not elicit any lymphoid activation or antibody formation, and likewise the entry of foreign antibody into the organism does not regularly cause any reaction to injury Probably the injury in bacterial disease potentiates the effect of the antigen on the lymphoid tissue and the antibody formation in analogy with the well known action of adjuvant and endotoxin This may explain the positive correlation found between the two kinds of reaction in bacterial disease

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 396

ON ESTIMATING THRESHOLD LIMITS FOR MERCURY IN BIOLOGICAL MATERIAL

BY

MATHS BERLIN

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This is a discussion and summary of the following eight papers

BERLIN M. (1962 a) A pump for use in measuring arteriovenous differences in concentration. Experientia 18 237

BERLIN M. (1962 b) Simultaneous measurement of blood flow glomerular filtration rate and urine secretion in the separate kidneys of anesthetized rabbits. Acta Physiol. Scand. 55 245

BERLIN M. (1962) Continuous recording of arteriovenous differences in concentration of radioactively labelled substances. Acta Physiol. Scand 56 82

BERLIN M. (1963) Renal uptake, retention and excretion of mercury II. A study in the rabbit during infusion of methyl and phenylmercuric compounds. Arch. Environmental Health.

BERLIN M. and GIBSON S. (1963) Renal uptake, retention and excretion of mercury I A study in the rabbit during infusion of mercuric chloride. Arch. Environmental Health.

BERLIN M. and ULLBERG S. (1963 a) Accumulation and retention of mercury in the mouse I An autoradiographic study after a single intravenous injection of mercuric chloride. Arch. Environmental Health.

BERLIN M. and ULLBERG S. (1963 b) Accumulation and retention of mercury in the mouse, II An autoradiographic comparison of phenylmercuric acetate with inorganic mercury Arch. Environmental Health.

BERLIN M. and ULLBERG S. (1963 c) Accumulation and retention of mercury in the mouse, III. An autoradiographic comparison of methylmercuric dicyandiamide with inorganic mercury Arch. Environmental Health.

Reference will be made to these papers using the years and letters as listed above in brackets.

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Maths Berlin

INTRODUCTION

Poisoning by mercury was described in China as long ago as 54 B C. and two hundred years later in India as well. The first known report of intoxication arising from the occupational use of mercury was given by Paracelsus on the basis of observations among the cinnabar miners at Idria (cited by ALMKVIST 1933) The appearance, in 1 00 of Bernardo Ramazzini s *De morbis artificum dia triba*" did much to elucidate the symptomatology of chronic mercury poisoning, being derived from a comprehensive study of illness among workers in the quicksilver mining, goldplating and mirror silvering industries, and among lay healers of that day whose practice consisted in the application of mercury ointment. Ramazzini's treatise was followed by several others in a similar vein these have all been reviewed by ALMKVIST The next important advance in our knowledge of industrial mercury intoxication was due to Adolph Kussmaul whose *"Untersuchungen über den konstitutionellen Merkurialismus"* was published in 1861 and summarized his observations, comprising 210 cases of chronic mercury poisoning, among workers at Fürt and Erlangen. Kussmaul pointed out, among other things, the difference between chronic ntoxication and the acute variety which was encountered as an undesirable consequence of the therapeutic use of mercury Several other studies followed, based on material from different industrial sites where exposure to mercury occurred these have been reviewed by NEAL *et al.* (1941) SPIEGL (195) and AXELSSON & FRIBERG (1960)

In contemporary industrial practice the use of mercury and its compounds is widespread. The element is employed for example, in various physical instruments and in electrical relays and in electrolytic cells and processes. The inorganic salts of mercury find use as catalysts in several chemical processes, and in the fur and felt hat industries. Several organic compounds of mercury because of their fungicidal and bactericidal properties, have been used as seed dressings and as preservatives for such organic materials as wood and wood pulp. In the past twenty years, a number of cases of occupational poisoning have been caused by these mercurials (HUNTER *et al.*, 1940 AHLMARK 1948 COTTER, 194) It has been proposed by several European and American authorities that the maximum allowable concentration (MA C.) in air for inorganic mercury as defined by the International Symposium on Maximum Allowable Concentration Values in Prague 1959 be 0 1 mg m³ air although Russian investigators have recommended a value considerably lowe i.e 0.01 mg/m air An

M.A.C. value of 0.01 mg/m³ air has been suggested for all organic mercurials.

AXELSSON & FRIBERG (1960) have reviewed the experiments and experience upon which were based the M.A.C. values suggested and applied in recent years. They concluded that the evidence in the literature did not permit more than a rather inexact estimate of the risks involved in occupational exposure to inorganic mercury and that an M.A.C. of 0.1 mg/m³ air was probably too high. They also pointed out that for organic mercurials, a scientific basis for the estimation of occupational hazard was lacking but that there were strong reasons to assume that the risk in chronic exposure could differ considerably between organic and inorganic compounds of mercury.

The urinary excretion of mercury has been used as an index in man of the degree of exposure to mercury and of the amount absorbed and retained in the body. TURRIAN *et al* (1956) however could not find any correlation between urinary excretion of mercury, clinical signs and level of exposure in a study of 58 workers exposed to mercury. They concluded that an excretion of more than 300 µg mercury per litre urine corresponds to an exposure considerably exceeding the M.A.C. value of 0.1 mg/m³ air. AXELSSON & FRIBERG (1960) have suggested that the lack of congruence between excretion and exposure shown in previous clinical investigations may be due, in part, to the difficulties in obtaining exact data in factory investigations of the level of exposure. They expected that if more accurate data were obtained, a better correlation could be demonstrated.

In view of the above it would be desirable, first, to establish a scientific basis for the determination of M.A.C. values for mercury and its compounds, and second, to find a reliable index of the degree of body retention of mercury which could be used in diagnosis and prophylaxis. To contribute to the achievement of these aims, the author has conducted a series of animal experiments in which the fate in the body of absorbed mercury was studied. In the discussion which follows, the results of this work, as well as other pertinent reports in the literature, will be summarized.

Theoretical considerations

It is well known from biochemical studies that mercury has a strong affinity for several organic substances or ligands common in biological structures. Of special significance are the sulfhydryl groups, as they occur widely and bind mercury with a very high association constant (HUGHES, 1957). Available biochemical data (summarized by PASSOW *et al* 1961) relating to the pharmacological and toxic effects of mercury and its

compounds indicate that they interfere in the biochemical reactions of the cell and the cell membrane It has also been shown in vitro that mercury in small concentrations inhibits a number of enzyme systems. It is reasonable to assume that there is some relation between the cell concentration of mercury and the degree of functional disorder which results. As the cell mercury concentration increases, cell function is disturbed. When this disturbance is of sufficient degree, the level of mercury can be then considered as the maximum allowable concentration for that cell. The degree of disturbance which constitutes this threshold is a matter of definition. One factor of importance here is the reversibility of the disturbance Chromosome damage, for example may be irreversible. In actual practice, however our ability to discriminate degrees of functional disorder is rather limited It seems practical to place the threshold at a point at which disturbances are detectable, which corresponds to the definition used for M.A.C. values and recommended by the Prague Symposium Probably this threshold will vary with the type of cell and the differences in the threshold between two cell types will depend upon, for example, the tendency of mercury to bind ligands and form inert complexes, thereby becoming more or less neutralized. Similarly it may be assumed that for every organ there is a threshold at which symptoms of functional disturbance appear and that this is dependent on what sort of cells the particular organ contains. How soon the highest tolerable concentration of mercury will be reached in an organ will depend upon the concentration to which it is exposed, and the rates at which uptake and elimination occur At a given level of exposure the threshold of functional disturbance is first reached in one or several organs, which may be designated the critical organ(s) for that exposure the tolerance of the organs in question is then the limiting factor in deciding how much absorption of mercury is permissible However it is possible that in different kinds of exposure, a different organ or organs will be critical. For example, an organ characterized by a very rapid uptake and a relatively rapid elimination of mercury may during a brief but severe exposure, rapidly reach its threshold value while an other organ showing sluggish uptake, pronounced retention and a relatively high sensitivity for mercury will not accumulate enough mercury during the short duration of the exposure to reach its threshold of functional disturbance At a lower level of exposure over a prolonged time however the threshold may never be attained in the first organ whereas it will be in the second, which in this case constitutes the critical organ.

To establish M.A.C. values for the human environment, one must know which organs accumulate and retain mercury how much mercury is retained and how sensitive these organs are to mercury Furthermore, the route by which mercury enters the body must be understood as this is dependent

upon the physical and chemical properties of the mercurial it is impor tant to know if the offending agent exists as a gas or as dust, in which case the particle size is critical or whether it is present in water or food. The factors determining the rate of body absorption will obviously vary in different cases. In the discussion which follows, only those factors *which are decisive in determining the* highest tolerable body burden of mer cury will be considered.

A great deal of information about the body distribution and retention of mercury under different conditions has been collected by experimental work in the past. This will be referred to below Very few experiments, however have been performed to estimate the sensitivity of different organs to mercury load. Our know ledge of this is derived mainly from the signs and symptoms of chronic and acute poisoning in animals and man, but the literature contains very few quantitative data As the clinical picture in mercury poisoning has been thoroughly reviewed by several authors, e.g. SPIEGL (1957) BATTI GELLI (1960) it will not be reiterated here.

It is of special interest to determine whether an index of the degree of body mercury load can be obtained by mea surement of the mercury concentra tion in body fluids in tissues which *can be readily biopsied, and in excreta.* To be useful an index must reliably reflect the concentration of mercury at least in the critical organs. From the points of view of clinical diagnosis and of occupational health it is also desi rable to have an indicator of the past history of exposure to mercury as well as of the present organ concentra tions. It has already been mentioned that the excretion of mercury in the urine has been employed as an index of body content. The limitations of this indicator will be elaborated below while the possibility of obtaining others which are more adequate will be considered.

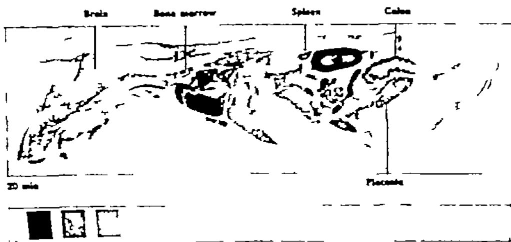
BODY DISTRIBUTION AND CUMULATIVE RISK

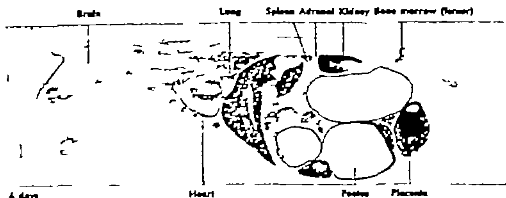
INORGANIC MERCURY

Body distribution

The body distribution of mercury arising from administration of the element or its inorganic compounds has been studied with a number of analytical techniques. The earliest used of these were chemical by which it was shown that mercury accumulates in the kidney liver spleen, intestine and myocardium (references to this work are given by BERLIN & ULLBERG, 1963 a) The largest deposits were invariably found in the kidney and after that, in the liver The order of concentration in the other organs has not been reported with much consistency no doubt as a result of differences in the mode of administration and dosage, as well as the relative insensitivity of the methods employed. More recently by the use of spectrographic methods or of radioactive isotopes mercury in small amounts has been demonstrated in other organs, for example in the brain (GRIFFITH *et al* 1954 FORBES *et al* 1954 HARRIS *et al* 1954) after its administration in subtoxic doses.

The aforementioned determinations were made on whole organs or large parts of them. When a histochemical technique or autoradiography was applied, however it was shown that the distribution of mercury in the kidney (ALMKVIST 1903 VOIGT 1958 BERGSTRAND *et al.*, 1958) and liver (FRIBERG *et al* 1957) is not uniform but rather quite differentiated. In the kidney mercury was largely concentrated in the proximal tubules and in the liver in the periphery of the lobules. BERLIN & ULLBERG (1963 a) made autoradiographs of 20 μ sagittal whole-body sections of mice given a single intravenous dose (0.5 mg mercury/kg body weight) of a highly active (1.5 C/g mercury) preparation of radiomercury The picture of body mercury distribution so obtained (Figs 1 and 2) emphasized the wide variation in concentration among and within the various organs and tissues In addition to the organs enumerated above mercury was also demonstrated in parts of the brain, in the skin, in the mucosa of the mouth, upper respiratory and alimentary tracts, and in bone marrow in the interstitial tissue of the testes (Fig 3) in the pancreas and salivary glands. In the brain (Fig. 4) an especially high concentration was found in the cerebellar cortex, parts of the hypothalamus, and the area postrema (in the posterior part of the floor of the fourth ventricle) the latter showing the highest concentration. The autoradiograms also confirmed earlier reports that mercury is excreted





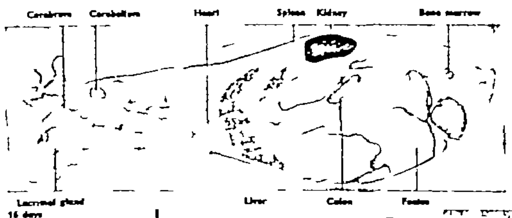


Fig. 1 Autoradiograms of sagittal whole-body sections of mice injected with $Hg^{203}Cl$ (0.5 mg Hg/kg body weight), taken 20 min, 4 days, and 16 days after the injection, from BERLIN & ULLBERG (1963). The autoradiogram of the isotope reference scale accompanying the 20 min section is shown. The radioactive intensity ratio between successive steps is 1/2.

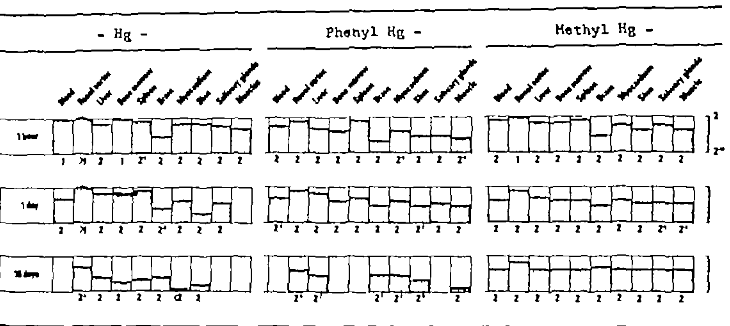


Fig. 3 The distribution of mercury among different organs at different times after single injections of 0.5 mg Hg/kg body weight as mercuric chloride (-Hg-) phenylmercuric acetate (Phenyl Hg-) and methylmercuric dicyandiamide (Methyl Hg-) respectively redrawn from BERLIN & ULLBERG (1963 a, b, c). The highest concentration in each organ measured by optical densitometry against an isotope reference scale is depicted as vertical bar on a geometric scale (common ratio 1/2) and is also given in arbitrary unit as a power of 2.



Fig. 3 An autoradiogram of a section of testis, 16 days after a single injection of $Hg^{203}Cl_2$ (0.5 mg Hg/kg body weight), in a mouse studied by BERLIN & ULLBERG (1963 a).

Fig. 4 An autoradiogram of a sagittal section of the brain of mouse, 16 days after a single dose of 0.5 mg Hg/kg body weight as $Hg^{203}Cl_2$, from BERLIN & ULLBERG (1963). Note the dense accumulation in the area postrema, which here lies just behind the caudal tip of the cerebellum, in the hypothalamus just anterior to the light field corresponding to the hypophysis, and in tissue adjacent to the ventricles. The olfactory lobe is also prominent in this section.

in bile and into the colon Autoradiograms of pregnant mice near term (18 days) revealed that very little mercury was transmitted to the foetus but that a great deal was accumulated in the placenta.

Risk of cumulative effects

The risk of mercury accumulation in the cells and tissue of the body is dependent on the relation between uptake and elimination. The rates of these processes have on the whole received little study FRIBERG (1956) however exposed rats to mercury for a prolonged time by repeated subcutaneous injection of radioactively-labelled mercuric chloride. When exposure was discontinued and an attempt made to exchange the accumulated mercury with inactive mercury large differences were found in the rate of exchange between radiomercury and inactive mercury in the kidneys, liver spleen and brain In the brain, no exchange was demonstrable during two weeks of trial. ROTHSTEIN & HAYES (1960) measured the retention of mercury in the rat after single intravenous or intramuscular injections of radiomercury at various times up to 48 days after injection Their results showed differences in the rates of elimination among different organs, and they concluded that the greatest retention and risk of accumulation were to be found in the kidney By densitometric comparison of the darkening in autoradiograms of whole-body sections of mice with that caused by appropriate isotope standards, BERLIN & ULLBERG (1963 a) were able to determine the order of mercury concentration within various organs at different times up to 16 days after a single intravenous injection of labelled mercuric chloride This technique gave an estimate of the comparative rates of elimination among different organs or parts of organs It was shown that those parts of the brain which took up mercury did so very slowly and that after the maximum concentration was reached, some time between the fourth and the sixteenth post injection day no elimination was detectable from them. Nor was there seen any elimination of the mercury taken up by the interstitial tissue of the testes. In the oral mucosa and the skin, retention was pronounced and elimination slow In the remaining organs, considerable elimination was observed once the maximal accumulation had been reached which, as a rule, occurred on the first day after injection. There is thus a considerable risk of accumulation of mercury in certain parts of the brain testes, skin and oral mucosa because of the very slow elimination of mercury from these sites, while there is a similar risk in the liver and kidney if the degree of accumulation in them in comparison to other organs is considered.

Discussion and conclusions

In a previous paper (BERLIN & ULLBERG 1963 a) it was pointed out that there is a conspicuous correlation between the site of accumulation of

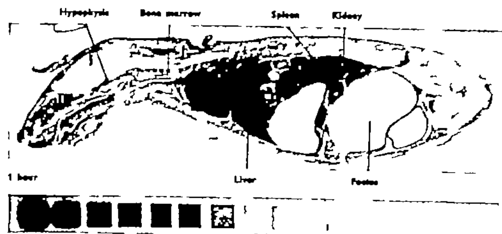
mercury as found in the mouse by autoradiography and the symptoms and signs of acute and chronic mercury poisoning The occurrence of a dense accumulation of mercury in the renal cortex is fully consistent with the dominance of renal signs in acute poisoning The colitis and other disturbances related to the intestinal tract, and the oral stomatitis which occur also correspond to mercury accumulation in the mucosa of the different parts of the intestinal tract. The slow uptake and elimination of mercury observed in autoradiograms of the mouse central nervous system may also explain, to some extent, the symptoms which arise in chronic mercury intoxication in man, provided that similar conditions obtain in the human central nervous system. The dominant symptoms are referable to the central nervous system and often appear with a certain delay and persist for a long time after exposure is withdrawn. It remains for future microautoradiographic studies to reveal if the accumulation of mercury in the brain is limited to certain types of cells, which is not unlikely

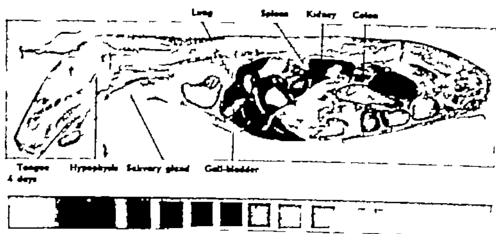
The distribution of mercury observed in the testes is very similar to that seen after cadmium exposure (BERLIN & ULLBERG 1963 d) In cadmium poisoning, testicular atrophy malformation of sperm and sterility have been observed (PARIZEK 1960) Pathological changes of this kind in chronic mercury poisoning have not been reported but the accumulation and retention of mercury which have been found in the testes suggest that further studies in this regard are warranted

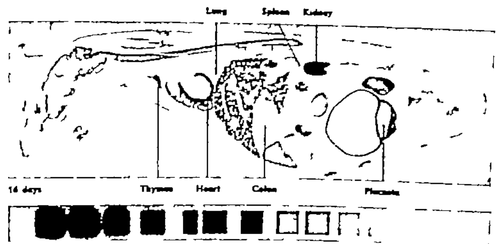
The commonest form of occupational exposure is to mercury vapour and most data referred to here are derived from studies in which mercury was given parenterally to experimental animals. However the results obtained after the administration of mercury as a vapour (ASHE *et al.*, 1953) do not differ significantly from those obtained after parenteral administration Moreover CLARKSON *et al* (1961) have clearly demonstrated that mercury vapour is readily oxidized in the blood to Hg ++ the process occurring mainly in the blood corpuscles There is, therefore no reason to believe that mercury entering the body as mercury vapour behaves differently compared to mercury injected in oxidized form

It is reasonable to assume on the basis of present knowledge, that the central nervous system and perhaps the testes are the critical organs in chronic exposure to subtoxic concentrations of mercury while in acute exposure to high concentrations, the kidney is probably the critical organ. It is possible however that there is some tissue in the body which is very sensitive to small doses of mercury but which does not show any marked accumulation even though serious

Fig 6. Autoradiograms of sagittal whole-body sections of mice injected with phenyl Hg^{203}-acetate (0.5 mg Hg/kg body weight), taken 1 hour 4 days and 16 days after injection from BERLIN & ULLBERG (1963 b). The autoradiogram of the isotope reference scale accompanying each section is shown. The radioactive intensity ratio between successive steps is 1/2.


Hypophysis
Bone marrow
Spleen
Kidney
1 hour
Liver
Foetus


Lung
Spleen
Kidney
Colon
Tongue
Hypophysis
Salivary gland
Gall-bladder
4 days


Lung
Spleen
Kidney
16 days
Thymus
Heart
Colon
Placenta

disturbances in its function may occur. At the present time however there is no clinical evidence to suggest the presence of tissue so sensitive. It is of some importance that further studies be made to determine the concentration of mercury in the central nervous system or its parts at which functional disturbances appear and to elucidate the mechanism by which mercury is taken up and accumulated there.

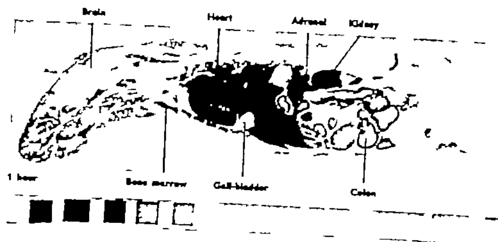
ORGANIC COMPOUNDS OF MERCURY

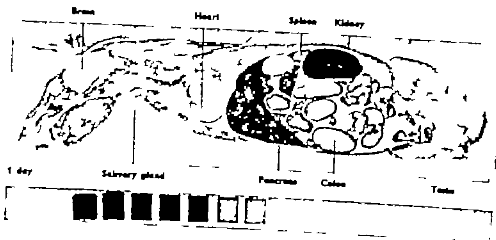
Body distribution

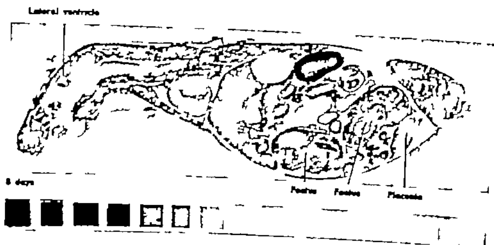
If an organic radical is attached to the mercury atom the biochemical properties as well as the toxicity of the latter are significantly altered. A striking illustration of this is found in the extensive work relating to the development of mercurial diuretics in the past half-century. Differences in toxicity and body distribution have also been shown for organic mercury compounds which are important in occupational health. PRICKETT *et al* (1950) found different body distributions of mercury after injection of phenylmercuric acetate and of inorganic mercury: accumulation was greater in the liver and more mercury was excreted in the faeces in the case of the organic compound. FRIBERG (1959) using the rat, and SWENSSON *et al* (1959) the dog, showed that mercury is excreted more slowly after administration of methylmercuric compounds than after inorganic mercury and that the body distributions differed as well. FRIBERG found more mercury in the brain after injection of a methylmercuric compound while the mercury accumulation in the kidney was less than after inorganic mercury.

BERLIN & ULLBERG (1963 b and c) using the autoradiographic technique referred to above studied the mercury distribution after a single intravenous injection of labelled aryl and alkyl compounds of mercury (phenylmercuric acetate and methylmercuric dicyandiamide, respectively). The dose in each case was 0.5 mg mercury/kg body weight. The distribution after injection of phenylmercuric acetate (Fig 5) was in several respects very similar to that found after injection of inorganic mercury but certain differences were noted. In the first 24 hours after injection of phenylmercuric acetate there was more accumulation in the liver and in the alimentary tract, but less in the renal cortex, than after inorganic mercury. These differences tended to disappear with time and, after 16 days, the distribution in the two cases were nearly identical. In the bone marrow and in the spleen however there was less retention in the case of phenylmercuric acetate.

Fig 6 Autoradiograms of sagittal whole-body sections of mice injected with methyl-Hg^{203}-dicyandiamide (0.5 mg Hg/kg body weight), taken 1 hour, 1 day and 8 days after injection, from BERLIN & ULLBERG (1963). The autoradiogram of the isotope reference scale accompanying each section is shown. The radioactive intensity ratio between successive steps is 1/3


Brain
Heart
Adrenal
Kidney
1 hour
Bone marrow
Gall-bladder
Colon


Brain
Heart
Spleen
Kidney
1 day
Salivary gland
Pancreas
Colon
Testis


Lateral ventricle
8 days
Foetus
Foetus
Placenta

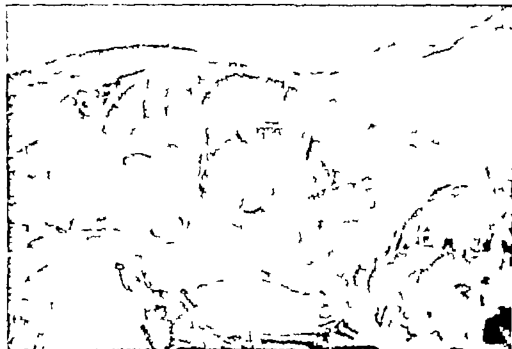


Fig 7 An autoradiogram of sagittal section of the brain of a mouse 16 days after a single dose of 0.5 mg Hg/kg body weight as methyl-Hg²⁰³-dicyandiamide from BERLIN & ULLBERG (1963). Note the dense accumulation in the hippocampus.

On the other hand the injection of methylmercuric dicyandiamide resulted in a body distribution of mercury (Fig 6) which was very different from that seen after injection of inorganic mercury There was a rather uniform distribution which extended to the foetus in the pregnant mouse Excretion of mercury could be observed in the bile and in the urine In the first 24 hours after injection the renal cortex and the liver accumulated more mercury than other organs, while very little mercury was seen in the brain and bone tissue. Afterwards, the mercury concentration in the liver tended with time to equal that of most other tissues in the body while mercury slowly accumulated in the brain to reach a maximum between the eighth and the sixteenth day after injection At that time the brain (Fig 7) especially the hippocampus and the cerebellar cortex showed a mercury concentration which was exceeded only by the renal cortex among the other body organs.

Risk of cumulative effects

The rate of elimination of mercury after injection of phenylmercuric acetate was found to be the same order of magnitude in most organs as in the case of injection of inorganic mercury as judged from mouse-section autoradiograms (BERLIN & ULL-

BERG 1963 b). The risk of accumulation in the brain should then be at least the same in exposure to phenylmercuric compounds as in exposure to inorganic mercury, while the risk must be considered greater in the liver, which has the higher uptake, and smaller in the kidney, which has the lower uptake, in the case of phenylmercuric acetate. In exposure to methylmercuric compounds, the risk of accumulation in the whole body is of quite another order than that after injection of inorganic mercury, as shown by FRIBERG (1959) and SWENSSON *et al* (1959). The very slow uptake and the dense accumulation of mercury in the brain demonstrated autoradiographically (BERLIN & ULLBERG 1963 c) are of special interest in view of clinical experience in methylmercuric poisoning, as neurological symptoms are found to persist for a long time after exposure ceases. Why the maximum accumulation of mercury in the brain occurs so late in relation to other tissues in the body remains unexplained.

Discussion and conclusions

It was expected from biochemical considerations that the body distribution of mercury after the injection of organic mercurials would differ from that after injection of inorganic mercury, provided that the organic compounds persist as such in the body for a significant time. The rather similar distributions found after administration of phenylmercuric acetate and inorganic mercury make it likely that the phenylmercuric group is broken down in the body. Such an interpretation is supported by the results of GAGE & SWAN (1961), who showed that inorganic mercury was excreted in the urine after injection of phenylmercuric compounds, and also by the results of MILLER *et al.* (1960), who found as inorganic mercury most mercury retained in the body one day after injection of a phenylmercuric compound.

The affinity of the methylmercuric radical for SH-groups is well established. On the basis of this property and the solubility of the methylmercuric derivatives, which renders them more diffusable through biological membranes, HUGHES (1957) postulated a uniform body distribution of mercury. The mercury distribution found in the mouse is consistent with this hypothesis and makes it unlikely that the carbon-mercury bond is broken in the body to any great extent.

The differences in distribution and elimination rate between the organic mercurials which have been studied confirm the expectation that it is not justified to look upon the organic compounds of mercury as a homogeneous group when deciding the highest tolerable body burden of mercury for each of them. Rather, it is necessary to consider the biochemical pharmacological and toxicological properties of each compound in estimating the tolerable body burden. No evidence has appeared which would justify a lower value for the body burden of mercury in exposure to phenylmercuric compounds than in exposure to inorganic mercury. The lower acute toxi

city which has been shown for phenyl mercuric compounds may be due to the fact that a large part of the absorbed mercury is retained in the blood and that the acute load in the kidney is small compared to what it is after exposure to inorganic mercury. The central nervous system seems to be the critical organ in chronic exposure to low concentrations of phenyl and methylmercuric compounds. It is also probable that for both these organic compounds, disturbances in kidney function can appear in chronic exposure. It is therefore important that the mercury load in the brain, kidneys and liver at which disturbances can be detected after exposure to organic mercury compounds, be determined by future investigation.

DIAGNOSTIC INDICES OF EXPOSURE TO AND RETENTION OF, MERCURY

The urinary excretion of mercury

INORGANIC MERCURY

Although it has long been known that mercury is accumulated in the kidneys and also excreted in the urine little is known of the renal mechanisms of uptake and excretion of mercury If the accumulation of mercury in the kidney is a part of the process of its excretion then the urinary excretion of mercury would be a good index of retention in the body since the kidneys are among those organs which retain mercury for the longest time As mentioned above, several authors have used the urinary excretion of mercury as an index of exposure to, and accumulation of mercury in man However the correlation between clinical findings and the urinary excretion of mercury is not convincing TURRIAN *et al.* (1956) made a special study of this problem and could not find any correlation between the two. In experiments with rats, FRIBERG (1956) found pronounced variations in the daily mercury excretion in the urine during a constant exposure to mercury ROTHSTEIN & HAYES (1960) studied the retention of mercury in the rat after a single injection by whole-body scintillation counting and also determined the mercury excretion in the urine Their results do not support the view that there is a direct correlation between mercury excretion in the urine and retention in the body By using a method (BERLIN 1962 a, b, c) which permitted in the rabbit, the continuous measurement of radioactive mercury in urine and in blood entering and leaving the kidneys, tongue) in the mucosa of the upper BERLIN & GIBSON (1963) determined the renal extraction, renal uptake and urinary excretion of mercury simultaneously with the measurement of the glomerular filtration rate by creatinine clearance. These measurements were made while the blood concentration of mercury was held constant by adjustment in the rate of infusion of labelled mercuric chloride During the four hours in which measurements were made, less than 10 % of the mercury in the blood was found to be extracted by the kidneys, and while up to 50 % of the infused mercury was accumulated in the kidneys, only a few per cent were excreted in the urine. The results indicated a correlation between the blood concentration and the urinary excretion of mercury at blood concentrations of the order found in occupational exposure to mercury (about 100 micrograms/100 ml) When plasma was ultrafiltered less than 1 % of the plasma mercury was found in the filtrate. It

therefore seems unlikely that plasma mercury passes through the glomerular membrane. The distribution of mercury between blood corpuscles and plasma was also studied during rising and falling blood concentrations of mercury. Variations in plasma and cell concentrations of mercury were out of phase indicating a slow turnover rate between the two compartments, each of which contained about half the blood total. These findings confirm the work of CLARKSON *et al.* (1961) who, in an independent study arrived at the same results with respect to the ultrafiltrability of mercury and the distribution of inorganic mercury between corpuscles and plasma.

The accumulation of mercury in the kidney can take place either directly from the blood or through tubular reabsorption of mercury. This problem was studied in rabbits by measuring the uptake of mercury in both kidneys, the ureter of one of which was ligated to prevent glomerular filtration. (BERLIN & GIBSON, 1963) It was shown that in spite of the fact that filtration in one kidney was prevented, the uptake of mercury was essentially the same in both, and it was concluded that mercury which accumulates in the kidney is for the most part derived directly from the blood.

All evidence indicates that mercury is excreted in the urine through tubular excretion and that the rate of excretion is related to the blood concentration of mercury. It is likely that the accumulation of mercury in renal tissue takes place independently of the process of mercury excretion and probably results from combination with ligands in the renal cells.

Conclusions

The evidence presented above does not support the view that the urinary excretion of mercury can serve as a reliable index of the accumulation of mercury in the body. Mercury excretion in the urine reflects the concentration of mercury neither in the kidneys nor in the brain. It is possible, however, considering the rather strong correlation between blood concentration of mercury and urinary excretion of mercury (BERLIN & GIBSON, 1963) that the urinary excretion of mercury could give an indication of the amount of mercury to which the body has been exposed in the very recent past. Nevertheless, experimental differences in the rate of excretion after different doses of mercury make it doubtful whether there is any linear relationship between the rate of excretion and the level of exposure.

ORGANIC COMPOUNDS OF MERCURY

It is known from investigations with mercurial diuretics that the combination of an organic radical with the mercury atom alters the rate and mode of mercury excretion in the kidney. Of the organic mercuric compounds which are of interest in occupational health, the phenyl and me-

thylmercuric compounds have been studied by PRICKETT *et al.* (1950) FRIBERG (1959) and SWENSSON *et al* (1959) It has been shown that the rate of mercury excretion is different after administration of each of these compounds and inorganic mercury This is especially clear in the case of methylmercuric compounds, which are excreted very slowly in the urine. There is evidence that phenylmercuric compounds are broken down in the body and are in large part excreted as inorganic mercury (GAGE & SWAN 1961) The technique referred to above which was used in studying the excretion of inorganic mercury has been applied to the problem of mercury excretion after infusion of methylmercuric dicyandiamide and of phenylmercuric acetate (BERLIN 1963) It was found that about 90 % of the blood mercury was bound to the corpuscles after infusion of each of these mercurials and that less than 1 % of the mercury in the plasma could be ultrafiltered. It was calculated that for equal plasma mercury concentrations, the mercury excretion after the phenylmercuric compound was of the same order as that after infusion of inorganic mercury while the excretion of mercury after infusion of the methylmercuric compound was about ten times less. In the case of both organic compounds and inorganic mercury there was considerably more mercury accumulated in the kidneys than was excreted in the urine The rate of urinary excretion of mercury after infusion of the organic compounds was also related to the blood concentration of mercury

Conclusions

The mechanism by which mercury is excreted in the urine after exposure to the aryl and alkyl mercury compounds which have been studied does not appear to differ in principle from that after exposure to inorganic mercury In the case of the phenylmercuric compound, the excretion of mercury reflects neither the body burden of mercury nor the concentration in the critical organ. In the case of the methylmercuric compound the blood concentration of mercury judged from autoradiographic investigations (BERLIN & ULLBERG 1963 c) is a better reflection of the body burden of mercury and hence in this case the renal excretion of mercury may be more significant of the body total However as the time course of mercury accumulation in the brain is out of phase with that of the rest of the body it may not be a useful indicator of the retention of mercury in the brain, which must be considered as the critical organ in exposure to methylmercuric compounds

Other Possible Indices

If the urinary excretion of mercury cannot serve as an index of the accumulation of mercury in the body can the mercury content of any other ex

cretion product or any tissue be used in this regard? The following requirements must be fulfilled by a suitable index. The specimen used for analysis must be easily obtained with little or no discomfort or risk to the patient. Its mercury content must be equal or reliably related to the mercury concentration in the critical organs. It is then natural to seek a tissue which accumulates mercury to the same degree as the brain or the testes and is accessible to biopsy. It is unlikely that other excreta such as faeces or saliva although readily obtainable can fulfill all requirements (BERLIN & ULLBERG 1963 a). In the case of inorganic mercury mouse autoradiograms (BERLIN & ULLBERG, 1963 a) showed accumulation of mercury in the skin in the epithelium of the mouth (especially the tongue) in the mucosa of the upper respiratory tract and colon. As activation analysis permits the determination with great accuracy of small amounts of mercury it seems probable that mercury could be determined by this method in biopsies of epithelial tissue. If mercury accumulates in the human skin to the same degree as in the mouse this tissue would be most suitable for biopsy and mercury determination. While the possibility that the skin may absorb mercury directly from the air or other contact has to be considered, there may be skin surfaces which are adequately protected. Scrapings from the plantar surface or toe nail clippings may be suitable. Scrapings from the tongue or biopsies of the upper respiratory epithelium may also be used but most workers are exposed to mercury vapour and therefore the mercury concentration at these sites may not reflect that in the critical organ. In hospitalized patients biopsies from the kidney liver or colonic mucosa may be employed to establish a correct diagnosis. In the case of the organic mercury compounds discussed here, there is no reason to assume any major differences between phenyl mercuric compounds and inorganic mercury when considering possible indices for the accumulation of mercury in the body. In the case of methylmercuric compounds, the period studied after the exposure in most investigations has been too short to permit any conclusions about the differences in elimination rate from different organs. It may be that in this case the blood concentration of mercury and also the mercury excretion in the urine can serve as suitable indices.

Conclusions

Present knowledge of the distribution and risk of accumulation of mercury in different organs of the body indicates that it would be worthwhile to study in victims of chronic exposure to mercury the mercury content in scrapings of the skin and tongue, in toe-nail clippings, and in biopsies of kidneys, colonic mucosa and liver and to correlate the findings with clinical signs of mercury intoxication.

SUMMARY

A brief historical review of the study of occupational exposure to mercury is presented and the basis for the present M.A.C. values of mercury compounds is examined. Important factors in the determination of the tolerable body burden of mercury are discussed, notably the body distribution of mercury after exposure and the risk of cumulation in different organs. In acute exposure the kidney and liver accumulate much mercury and are hence liable to injury, while recent findings indicate that in chronic exposure to moderate levels of mercury the brain and possibly testes are the critical organs because of a pronounced tendency to cumulation. The possibility of obtaining an index of mercury retention is explored. It is concluded that urinary mercury excretion does not reflect the level of body retention although it may indicate very recent exposure. It is suggested that mercury concentration in biopsies of skin, liver, kidney and colonic mucosa may serve as an index of body retention of mercury.

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SUPPLEMENTUM 397

INITIAL HEPARIN THERAPY AS A SUPPLEMENT TO PERORAL ANTICOAGULANTS IN ACUTE MYOCARDIAL INFARCTION

BY

ERIK ENGER ALF CHR JULSRUD an[illegible]
KNUT KIRKE[illegible]

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FROM MEDICAL DEPARTMENT VII, ULLEVÅL HOSPITAL,
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Initial Heparin Therapy as a Supplement to Peroral Anticoagulants in Acute Myocardial Infarction

BY

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Contents

Introduction

The first evidence of the benefit of anticoagulants in myocardial infarction was given in 1939 by Solandt *et al.*, who showed that heparin could hamper coronary thrombosis experimentally in dogs and prevent the development of mural thrombosis over an area of injured cardiac muscle. During the following years, developments of reliable and inexpensive peroral anticoagulants, the effect of which could be controlled with a reasonable degree of safety by relatively simple methods, opened the field of large scale short- and long-term anticoagulant therapy in human medicine.

In the early 1940s scattered reports appeared favouring the use of anticoagulants in acute myocardial infarction. During the last 15 years an extensive literature has accumulated on this subject. Most investigations have yielded evidence favouring the use of anticoagulants in this condition. But reliable reports have also appeared expressing profound doubt of their value. Three years ago, however, at the start of the present study, anticoagulants were probably used in the treatment of patients with an acute coronary occlusion in most hospitals in Western Europe and in the U.S.A. Last year the report appeared of the extensive Copenhagen study performed by Hilden *et al.* Their conclusions were definitely negative as to the value of anticoagulants in the treatment of acute myocardial infarction. It is, however, characteristic of the present conflicting situation that although there have been only few Scandinavian opponents to the design of the Danish study and to the interpretation of the results obtained, the use of anticoagulants is still common practice in these countries.

There are various reasons for our ambiguous attitude to this matter. It seems to be a natural course of events that medical discoveries with potential benefits to our patients arouse enthusiasm and emotion that often outstrip human logic. No doubt this is responsible for the prevailing custom of applying a new drug first and evaluating it later. Faulty technique in the study of the effect of anticoagulants in acute myocardial infarction is responsible for the unhappy fact that despite many years of clinical experience, the question as to the value of these drugs is still undecided. It seems therefore appropriate to deal in some detail with the requirements which ought to be fulfilled when studying the effect of anticoagulants in the treatment of myocardial infarction.

CHAPTER I

The Purpose of Using Anticoagulants in Acute Myocardial Infarction and some Notes on the Means by which this Treatment should be Studied and Evaluated

The generally accepted purposes of using anticoagulants in acute myocardial infarction are:

1. to prevent distal and retrograde thrombosis of the coronary arteries,
2. to prevent the development of mural thrombi and the associated risk of systemic embolization, and
3. to prevent peripheral thrombophlebitis and the associated risk of pulmonary embolism.

Theoretically the rational foundation of this treatment is sound. The myocardial infarction is usually the product of a thrombotic process. By using ordinary or refined methods an increased coagulability of blood can often be demonstrated during the early phase of coronary occlusion (Ogura *et al.* 1946, Rosenthal *et al.* 1952, Beaumont *et al.* 1953, Gormsen 1959). The conditions of shock, cardiac arrhythmia and congestive heart failure which often complicate the condition, are associated with an increased tendency to thrombo-embolism. Finally the risk of developing venous thrombosis is increased during the first weeks of strict bed-rest.

It is important to realize that the only of this treatment is to reduce the coagulability of blood. Therefore, the essential information which should be presented in a study of anticoagulants is whether a satisfactory level of reduced coagulability of blood actually was achieved. Thereafter the interest may be focused on the thrombo-embolic complications occurring. The documentation of crude mortality rates may often be fallacious. Presentation of such figures is worthless if not associated with close information on the clinical manifestations and the autopsy findings of thrombo-embolism.

Usually the effect of anticoagulants in acute myocardial infarction is studied by comparing the mortality rate and the incidence of thrombo-embolic complications in a series of patients given this therapy with a non-treated control group. In every other respect the medical care of the groups should be essentially equal. The immediate prognosis of patients with an acute myocardial infarction is influenced by a number of factors. It is essential that these are equally distributed between the two groups. Both series should be demonstrated comparatively with respect to age, sex, pre-existing hypertension, pre-existing angina pectoris, incidence of previous myocardial infarctions, obesity myocardial state prior to the infarction,

duration from onset of symptoms to admission to hospital, degree of shock at the onset and incidence of heart failure. The fulfilment of these requirements will involve a fairly large number of patients and above all a highly unbiased distribution of cases between the groups. The latter is the crucial point which too often constitutes the weak link in an otherwise well planned and conducted study. There exist reports from different hospitals and from different periods of time giving mortality rates of patients with acute myocardial infarction not treated with anticoagulants ranging from 3.5 to 78 per cent (vide infra). It is therefore worthless to compare treated and non-treated series of patients originating from different geographic areas or from different periods of time. Recently it has been demonstrated that even the matching of two patient groups from different departments of the same hospital admitted during the same period may provide fallacious results (vide infra.) Thus, the principle of unity of time and place is imperative in investigations of this problem. The distribution of patients between the groups should be entirely unbiased and the original scheme of allocation should be strictly adhered to throughout the study.

A very difficult, but pertinent question is whether the treatment actually given should be concealed from the patient and from the medical personnel responsible for the immediate medical care, i.e. whether the study should be conducted along the lines of the double blind technique. This is an ideal requirement in most clinical trials. It should be realized that even with objective measurements the subconscious bias of the investigator may affect the answer if he knows which patient is being treated and which is not. This is especially true with the diagnosis of thrombo-embolic complications, where the criteria sometimes are not clear-cut. When seeing a patient with a few rales at the basis and vague shadows in the chest roentgenogram, the physician may be influenced in his decision as to whether or not the patient had a pulmonary embolus, by the knowledge that the patient did or did not receive an anticoagulant. It may be argued that most studies largely deal with mortality rate. Inasmuch as death is a decisive end point, the factor of subconscious bias ought to be unimportant. However, as has been emphasized previously, an essential point is whether the death in some way may be ascribed to a thrombo-embolic complication or to a complication related to the reduced coagulability of blood, i.e. to hemorrhage. Undoubtedly the final decision made might be influenced by the subconscious bias of the clinician or the pathologist.

Another point favouring the principle of double blind technique is the emotional status of a patient. A treated patient in any study is likely to receive extra attention from the medical personnel coming in contact with him during hospital stay. This extra attention may tip the balance favourably in some patients. In this respect it is interesting that blood coagulability may be influenced by emotional factors (Schneider 1951, Macht 1952, Friedman *et al.* 1958). Therefore, it is appropriate to consider the psychological status of the patients in studies which do not employ the double blind technique. Most patients with myocardial infarction experience fear and anxiety, sometimes of great intensity and duration. However, the knowledge that he is receiving a new, potentially life-saving drug in which his physician has great faith, may well relieve much of his fear and anxiety and thus reduce the coagulability of his blood. Therefore, the failure to control adequately the subconscious bias of the investigator and the emotional status of the patient might well result in serious

errors. However to our knowledge there has been no report in the literature of any larger study of this problem using the double blind technique. And admittedly enormous practical difficulties will be met if this principle is introduced in large-scale investigations on the effect of anticoagulants in acute myocardial infarction. In the present study the heparin treatment was made 'single blind'. The purpose was to counterbalance the emotional tension of the patients related to the intravenous injections. No attempt was made to introduce the double blind technique, mainly because the subcutaneous after-bleedings which regularly occurred at the injection sites of heparin would have revealed to the observer the group to which the patient belonged.

When evaluating the results of a study on the effect of anticoagulants in acute myocardial infarction, primary attention should be paid to the distribution of patients between the groups. It should be clearly demonstrated that this process was essentially unbiased and strictly unchanged throughout the study. The two groups should be comparable with respect to the above-mentioned factors known to influence the prognosis in patients with an acute myocardial infarction. The clinical criteria of thrombo-embolic manifestations should be defined and details presented regarding the patients in whom these complications occurred. The anticoagulant effect actually obtained should be noted with special reference to the prothrombin level at which thrombo-embolism or bleeding episodes occurred. In fatal cases the possible relationship to thrombo-embolic complications or bleedings should be evaluated. This may well be a difficult task. It is usually impossible to decide whether there has been an extension of the thrombus within the coronary three secondary to the initial thrombotic process. Finally the following parameters should be subjected to statistical analysis: 1) the incidence of thrombo-embolic complications and bleeding episodes in the two groups, and 2) the mortality rate from these complications. The validity of differences between two groups of patients in a clinical trial is proved by applying a statistical significance test, i. e. to determine the probability of having obtained the result by chance. In clinical trials the confidence limit usually required is 5 per cent, i. e. a difference between two parameters is accepted as real and valid if the probability of having obtained the result by pure chance is less than 1 in 20. It is essential for clinicians to realize that this is a way of applying well-defined mathematical methods to ill-defined biological materials. This approach is introduced to compensate for the belief and the prejudice of the clinician, but may also well conceal factors or facts of clinical significance. Thus, a therapeutic programme may well be of some clinical value, if only slight, although no results of statistical validity can be demonstrated because the number of patients compared is too small. But to the patient whose death is prevented by this treatment and to his family and to his doctor the expression 'without any statistical significance' has no meaning. Therefore, an essential requirement in clinical trials is that the study should comprise a reasonably large number of patients. This also applies to investigations on the effect of anticoagulants in acute myocardial infarction. It has been claimed that such studies should involve several hundreds of patients. It may be argued, however, that if an enormous material is needed to establish the effect of a therapy, its clinical value may be outweighed by practical, administrative or economic reasons.

Finally the total material should be of a sufficient magnitude to permit statis-

tical analysis of the effect obtained in selected groups of patients. For instance, it may be of great interest whether there exists any disparity of this effect in the two sexes or in different age groups.

Admittedly enormous practical difficulties are met with during the performance of a study on the effect of anticoagulants in acute myocardial infarction if all of the above-mentioned requirements are fulfilled. A clinical trial along these lines demands the utmost medical skill, exactness and patience. To our knowledge the ideal study on this problem has not yet appeared. Nor do we ourselves claim to offer such a study here. However the considerations given in this chapter form the base of our subsequent survey of the literature and are the key to the critical notes on these papers and on the present one.

CHAPTER II

Previous Studies on the Effect of Anticoagulants in Acute Myocardial Infarction

During the past 15 years an extensive literature has accumulated on the use of anticoagulants in acute myocardial infarction. In this chapter some well-known papers appearing in the Anglo-American and Scandinavian literature are reviewed. The selection and the presentation are as unbiased and unprejudiced as possible. Papers favouring the use of anticoagulants and those unable to demonstrate any effect from them are grouped separately

Studies yielding evidence of an effect of anticoagulants in acute myocardial infarction

Glueck et al. (1948) reported on 25 patients with acute myocardial infarction who were given combined heparin and Dicumarol therapy The control group of 25 patients was obtained by omitting anticoagulants in every other patient. There were 8 deaths in the control group as compared to 3 deaths in the treated group. Thrombo-embolic complications occurred in 6 of the control cases and possibly in one of the treated cases. There is deficient information on the comparability of these small series of patients and the autopsy rate is low

In 1948 appeared a preliminary report of the extensive study from the American Heart Association's Committee that was appointed under the chairmanship of *I S Wright* to evaluate anticoagulants in the treatment of coronary thrombosis with myocardial infarction. The final presentation was given in 1954 From a quantitative point of view this extensive, cooperative clinical trial is superior to other

studies on this subject. The study was conducted in 16 American hospitals and involved more than 1,000 patients. Patients admitted on odd-numbered days were given anticoagulants, while those admitted on even-numbered days were not. Heparin was administered to 15 per cent of the patients in the treated group. The remainder received peroral anticoagulants only. In all important respects the groups were comparable. The mortality rate of the control group was 23.4 per cent against 16.0 per cent of the treated group. The difference was statistically significant. In the control group 26 per cent of the cases developed thrombo-embolic complications compared with 11 per cent in the treated group. The incidence of pulmonary embolism was reduced by half. At autopsy there was a marked difference in the incidence of thrombo-embolic complications. These results are impressive. However, several objections exist to this study. Although the initial plan called for the alternate case method, a great proportion of patients were treated out of turn. The reason for this is not completely understood. Thus, it is impossible to decide whether some kind of selection was made which might have influenced the results obtained. Unless one is familiar with the American hospital system it is also difficult to evaluate the significance of the difference between patients admitted to open wards and to private beds. The mortality rate of the *treated ward* cases was 19 per cent, while that of the *control private* cases was 20 per cent. One might therefore deduce that changing a patient from ward to private status is as effective as anticoagulant therapy in reducing mortality. Thus serious doubt is introduced with respect to the homogeneity of this enormous case material. This applies to the selection of cases between the groups as well as to the anticoagulant therapy and the medical care given. In addition there is insufficient information on the principle by which additional heparin treatment was administered. Finally, little attention was paid to the fact that there was no difference in the mortality rate for patients below 59 years old, the benefit from anticoagulant therapy thus being confined to older and more complicated cases.

Schilling (1950) favoured the use of anticoagulants in all cases of myocardial infarction. Sixty patients of one medical department received anticoagulants while an equal number of patients admitted to another department of the same hospital served as controls. Dicumarol was used as the principal anticoagulant. In some cases heparin was added. To patients who were critically ill heparin only was administered during the initial phase. The groups were comparable in regard to age and sex but no further comment is presented on the distribution of other clinical features between the groups. In the treated group 10 patients died compared to 24 patients in the control group. In the treated group there was no fatality primarily due to a thrombo-embolic complication, whereas this happened in 11 cases of the control group. Five per cent of the treated cases experienced a thrombo-embolic complication compared to 25 per cent of the control group. There was no difference in the mortality rate and the incidence of thrombo-embolism between the group given combined heparin and Dicumarol therapy and that given peroral anticoagulants only. These groups, however, were scarcely comparable, since heparin was used predominantly in severely ill patients. Finally, serious doubt of the validity of the results obtained is introduced by the following statement: "... when obvious necessity for anticoagulant therapy occurred in the control series, these patients received such therapy."

Tulloch & Gilchrist (1950) studied a total

of 154 patients with acute myocardial infarction in the same ward. The patients were distributed between the groups according to the day of admission. The treated series received Dicumarol and an initial course of heparin by intravenous injections until a prothrombin content below 30 per cent of normal was achieved. The groups were found comparable with respect to a great number of clinical features. After excluding patients who died within the first 6 hours of admission, the mortality rate of the treated series was 22.8 per cent as against 40.5 per cent of the control series. The difference was statistically significant. When the sexes were considered separately the difference in the mortality rate was of statistical significance for the males only. In the control series 28 per cent developed a thrombo-embolic complication against 13 per cent in the treated series. Fourteen patients in the control series died from thrombo-embolism compared to 4 patients in the treated series. This study showed that the proper use of anticoagulants can halve the mortality rate and the incidence of thrombo-embolic complications in myocardial infarction. Apart from a fairly low autopsy rate serious objections do not exist to this study.

Holten (1951) reported on an extensive, co-operative study undertaken by 21 clinics in Denmark. The alternate day method was used for the allocation. The treated patients were given Dicumarol and an initial course of heparin. The treated group consisted of 174 patients, while 256 patients served as controls. After excluding the fatalities occurring within the first 24 hours of admission, the mortality rate of the treated series was 22.5 per cent compared with 36.0 per cent of the control series. The difference was statistically significant. The reduction in the mortality rate below 60 years of age was less pronounced than for older age groups. Unlike that in the results of Wright *et al.*, however the difference was still of statistical significance. Four per cent of the cases in the treated group experienced thrombo-embolic complications as against 14 per cent in the control group. This difference is also of statistical significance. Several objections exist to this study. Above all, a great number of uncontrolled factors are introduced in a study involving so many hospitals and doctors. This is probably the explanation of the great difference in the number of patients included in the groups. It must have been a fairly pronounced deviation from the original design for the distribution of patients between the groups. The evidence presented on the comparability of the groups is not complete, and there is also deficient information on the autopsy findings. Finally, according to present-day knowledge, the anticoagulant scheme employed was insufficient.

Manchester & Relkin (1954) studied 300 patients with acute myocardial infarction at home and in hospital. The alternate case method was used for allocation between the groups. Seventy-two per cent of the treated cases were given Dicumarol associated with an initial course of heparin, while the remaining cases were given peroral anticoagulants only. The series were found comparable with respect to a number of clinical features. The mortality rate of the treated group was 12 per cent compared to 28 per cent of the control group. The difference was statistically significant. Thrombo-embolic complications occurred in 17.5 per cent of the cases in the control group as against 6 per cent in the treated group. Although the groups were demonstrated comparable in regard to many relevant factors it may be argued that this case material must have been fairly heterogeneous. Thus some patients were ob-

served in their homes and others in hospital. Some patients were seen a few hours after the acute attack while others were included several days later. No account is presented on the principle by which heparin was administered to some patients. Nor is there any information on the prognosis of the patients on different treatment schemes. Finally autopsy findings are not evaluated.

Richards (1958) studied the outcome of patients with acute myocardial infarction in the same hospital before and after the introduction of anticoagulants. No change of the mortality rate could be demonstrated. *Mc Clukie & Seaton* (1959) compared the mortality figures of patients with acute myocardial infarction over a period of 4 years at two medical units of the same hospital as that of Richard's study. In one unit peroral anticoagulants were used and in the other they were not. The mortality rate of the treated series was 19 per cent compared with 29 per cent of the control series. The difference is of statistical significance. These conflicting results prompted another study at the same units, an identical scheme for anticoagulant therapy now being employed in both (*Richards* & *Seaton* 1961). The mortality rate of the unit at which anticoagulants not had been used previously was still significantly higher (34 per cent) than that of the other (24 per cent). The authors were able to demonstrate that this difference might have been related to a slight but definite tendency to admit patients more critically ill to the unit with the higher mortality figures. Information which has been previously indicated as important is lacking from these reports. They are, however extremely interesting in their demonstration of the paramount importance of using the principle of unity in place and time when performing such studies.

Gumpert (1962) made a retrospective study on 104 male patients with acute myocardial infarction. Some of these had no anticoagulants, whereas the majority received various forms of anticoagulant therapy. When the death rate within the first 3 days from the onset of cardiac pain was considered, there was a difference of statistical significance in favour of combined Dicumarol/heparin treatment. However the various groups of patients were small and serious doubt exists with respect to their comparability. Finally important information on a great number of clinical factors is omitted from the presentation.

Studies unable to demonstrate any effect of anticoagulants in acute myocardial infarction

Feldman et al. (1952) performed a controlled clinical study in a total of 152 patients. The alternate case method was used for distribution between the groups. Dicumarol was used as the principal anticoagulant, while heparin was added in cases where an immediate anticoagulant effect was deemed necessary. The groups were found comparable in regard to a great number of clinical factors. The mortality rate of the groups was identical, namely 30 per cent. Thrombo-embolic complications occurred in 4 patients of the treated series and in 6 patients of the control series. This study therefore, does not favour the routine use of anticoagulants in acute myocardial infarction. Some doubt exists, however with respect to the homogeneity and the comparability of the groups. Thus, one-third of the treated patients were included in the study as late as between the 2nd and 10th day after onset of the acute attack. There is no information on this point for the control series. Neither is there any information on the comparability of the groups with respect to the

severity of illness on admission to hospital. The principle for the additional use of heparin is not clearly stated. Finally there is no report on autopsy findings.

Schnur (1953) gathered information on 1,300 patients with acute myocardial infarction admitted to several American hospitals during a 10-year period who were not subjected to anticoagulant treatment. In addition he presented major statistical objections to the study by *Wright et al.* Schnur concluded that incontrovertible proof as to the advantage of using anticoagulants in patients mildly ill on admission to hospital had not been presented so far. However so many statistical objections exist to such a study that the validity of the conclusions are highly questionable.

Russek & Zohman (1957) proposed the significance of classifying patients with acute myocardial infarction in good- and poor-risk cases according to the presence of clinical signs known to imply an unfavourable outlook. The authors observed 511 good-risk patients to whom anticoagulants were not administered. The mortality rate and the rate of thromboembolism of this series were 3.5 per cent and 3.7 per cent respectively. These results are in marked contrast to the very much higher figures for mortality rate and thrombo-embolic complications reported from unselected series or from materials consisting mainly of poor-risk cases. The authors maintain that the use of anticoagulants in good-risk cases is more dangerous than the risk of thromboembolic complications. However it may be argued that the proper use and control of anticoagulant therapy involve a minimal danger of serious hemorrhagic complications. Furthermore, it is common clinical experience that it is extremely difficult to foresee the outcome in the individual patient with an acute myocardial infarction on admission to hospital. Therefore, the only reasonable time to classify an attack of coronary occlusion as mild is when the patient is discharged from hospital in good condition.

Carleton et al. (1960) made an almost ideal study by the double blind technique on the effect of intravenous heparin for 4 weeks as the only anticoagulant agent in a total of 81 patients with acute myocardial infarction. The patients were randomly assigned to the two series and these were proved comparable with respect to a great number of clinical features. The mortality rate of the treated series was 22 per cent and of the control series 25 per cent. Thrombo-embolic phenomena developed in 10 cases of the heparin series and in 12 cases of the control series. Hemorrhagic complications occurred in 9 cases of the heparin series. None of these were fatal. Apart from the relatively small number of cases involved, no serious argument exists against this study which indicates that heparin is without benefit during convalescence from acute myocardial infarction.

Myers & Bauer (1961) presented a study on the use of anticoagulants in relatively young males with acute myocardial infarction admitted to an army hospital. Fifty patients received peroral anticoagulant therapy and 50 patients served as controls. The average age of both series was 44 years. The mortality rate of the total series was 3.8 per cent. When patients who died within the first 24 hours of admission were excluded, there was one fatality case only. This patient belonged to the control series. In the treated series 18 per cent developed thrombo-embolic complications compared with 16 per cent in the control series. There was no death due to thrombo-embolism. This study clearly demonstrates the favour

able outlook of young males experiencing their first myocardial infarction. Thus, these results emphasize the importance of controlling factors like sex and age when studying the effect of anticoagulants in acute myocardial infarction. In some respects the presentation of this study is incomplete. This applies to the comparability of the groups and to the details of the anticoagulant regime actually performed, especially in patients experiencing a thrombo-embolic complication.

Hildén et al. (1961) studied 1 404 patients with acute myocardial infarction admitted to 4 medical departments in Copenhagen during a 4-year period. Each department used Dicumarol combined with initial heparin therapy for 2 of the 4 years. In the treated series 371 cases remained for final evaluation, while the control series comprised 429 cases. The two series were comparable with respect to a number of clinical features. After excluding the fatalities within the first 48 hours of admission the mortality rates in the treated series and the control series were 23 per cent and 25 per cent respectively. Ten per cent of the cases in the treated group and 14 per cent in the control group developed clinical signs of thrombo-embolism. The difference was not statistically significant. However when the thrombo-embolic manifestations discovered at autopsy were added to those observed clinically the rate of thrombo-embolism in the treated series was 15 per cent compared with 25 per cent in the control series. This difference was of statistical significance ($p = 0.01$). Furthermore, in the treated series the mortality rate from thrombo-embolism was 1.4 per cent compared with 4.0 per cent in the control series. This difference was also of statistical significance ($p = 0.04$). Finally bleeding episodes were encountered in 19 per cent of the cases in the treated series. Most of these episodes were mild, but death ensued in 4 cases. There was no information on the time at which the bleedings occurred, i. e. it is impossible to decide whether they were related to heparin or to the peroral anticoagulant therapy. The authors stressed the fact that the mortality rate was uninfluenced by the use of anticoagulants, and concluded that this treatment was not indicated in acute myocardial infarction. Several objections may be raised to this study. The uncertainty introduced by comparing case materials from different hospitals and from different periods of time has been emphasized previously. Furthermore, the intensity of the peroral anticoagulant therapy actually performed is not in accordance with the currently favoured requirements for such treatment. Finally the conclusions of this study may be seriously questioned. The essential aim of anticoagulant therapy is to reduce the incidence of thrombo-embolic complications. In the Danish study a significant reduction in the mortality rate from thrombo-embolic complications was demonstrated. It seems unfair to conceal this effect, although small, behind over all mortality figures which above all reflect that the grave prognosis of acute myocardial infarction is mainly uninfluenced by any treatment. If the Danish results are interpreted in favour of the use of anticoagulants, it is tempting to relate their effect mainly to the heparin treatment, since the peroral anticoagulant regime was partly insufficiently conducted.

The most striking experience from this literature survey is the great variability of the various studies in regard to the mortality rate and the incidence of observed thrombo-embolic complications. The designing, the performance and the presentation of most studies by no means satisfy the requirements set forth in chapter I. This is the

obvious explanation of the pronounced deviation of the results obtained with respect to the value of anticoagulants in the treatment of patients with acute myocardial infarction. In our opinion the objections to studies unable to demonstrate any effect of anticoagulants are stronger than those which can be raised to those favouring their use. Favourable reports seem to have outnumbered the unfavourable and the cautious. These have shown that the main benefit to be expected from this treatment is the prevention of further thrombo-embolic episodes, especially venous thrombosis and subsequent pulmonary embolism. The rate of hemorrhagic complications shows the same great variability with figures ranging from 1 to 20 per cent. This is probably due to differences in the planning the performance and the control of the anticoagulant regime. With the purpose of the present study in mind it is essential to realize that nothing can be learned from these studies with respect to the relative importance of heparin versus peroral anticoagulants in acute myocardial infarction. This applies to the mortality rate, to the incidence of thrombo-embolism, and to hemorrhagic complications. In most studies peroral anticoagulants have been combined with an initial course of heparin. In others the peroral route only has been applied, and finally heparin has been used as the sole agent in some studies. From statements previously made, however it is clear that it is a grave mistake to draw conclusions from studies obviously not comparable in a great variety of important features.

Chapter III

Previous Studies on the Effect of Heparin versus Peroral Anticoagulants in Acute Myocardial Infarction

Heparin has more rapid and profound action on the coagulability of blood than peroral anticoagulants. It is widely accepted that the appropriate approach for anticoagulant therapy in acute myocardial infarction is to combine peroral anticoagulants with intermittent heparin injections until a sufficient reduction of the prothrombin content of plasma has been achieved. The significance of early heparin treatment is especially emphasized in patients already presenting signs of peripheral thrombo-embolism, in those critically ill, and when nausea makes peroral therapy difficult. On the other hand, heparin treatment involves an increased risk of hemorrhagic complications; and the frequent injections are inconvenient to the patients and laborious for the medical personnel. Finally heparin is far more expensive than peroral anticoagulants.

In the extensive literature on the clinical use of anticoagulants there are few reports concerned specifically with the value of he

parin. From the papers reviewed in chapter II it is difficult to assess the separate value of using heparin. In the studies by Glueck *et al.*, Tulloch & Gilchrist, Holten, and Hilden *et al.*, heparin was used routinely in association with peroral anticoagulants until a satisfactory reduction of the prothrombin level was achieved. In the study by Wright *et al.* heparin was administered to 15 per cent of the treated group, preferably to those who were critically ill on admission. Feldman added an initial course of heparin to cases where an immediate anticoagulant effect was deemed necessary. Schilling made a similar approach. He was unable to demonstrate any difference in the mortality rate and the incidence of thromboembolism between the group given combined Dicumarol/heparin treatment and that given peroral anticoagulants only. However these groups were not comparable. Most of these studies give deficient information on important aspects of the anticoagulant treatment. This applies especially to the time at which the thromboembolic complications occurred and to the anticoagulant effect actually obtained at this moment. Therefore, these studies are without significance in assessing the value of early heparin treatment. One may however suspect that the beneficial effect obtained by anticoagulants in many of these studies might have been attributed to a considerable degree to the initial heparin therapy. This might have been the case in studies where heparin was reserved for patients who were critically ill and in studies where the achieved intensity of the peroral anticoagulant treatment did not tally with the currently favoured requirements. Similar considerations may be applied to the observed incidence of hemorrhagic complications. It is usually impossible to deduce from the various papers whether the bleeding episodes might have been related to heparin or to the peroral anticoagulant regime.

There exist two studies concerned with the isolated use of heparin contra peroral anticoagulants in acute myocardial infarction. *Griffith et al.* (1957) treated 92 patients continuously with subcutaneous heparin injections and 72 patients with Dicumarol. Originally they planned to assign the patients alternatively to the two groups. During the study however they were impressed by the efficiency of heparin and several patients were therefore given heparin out of turn. This is the reason why 20 per cent of the cases in the heparin series experienced shock on admission to hospital as against 8 per cent in the Dicumarol group. The frequency of severe cardiac arrhythmia and congestive heart failure was also significantly higher in the heparin group. Thrombo-embolic complications occurred in 2 cases of the heparin group and in 1 case of the other. Despite the impressiveness of these results it is difficult to assess their real value. The presentation is short and in several respects incomplete. No details are presented on the peroral anticoagulant therapy performed. Nor is there any information on death rates or autopsy findings. However the results are strongly in favour of the assumption that heparin is superior to Dicumarol as an anticoagulant in acute myocardial infarction.

The study by *Manson & Fullerton* (1956) seems to indicate the opposite conclusion. These authors studied two series of patients admitted to two different units of the same hospital. At one unit 102 patients received peroral anticoagulant treatment. Sixty-two patients admitted to the other unit were given 12,500 units of heparin twice daily for 3 weeks. These two groups were compared with 150 patients admitted to another hospital with acute myocardial infarction who were not given anticoagulant therapy during the same period. It was maintained that the three groups of patients

were comparable. The death rate of patients treated with peroral anticoagulants was 7.8 per cent and thrombo-embolic complications occurred in 20.6 per cent of the cases. The mortality rate in the heparin series was 24.2 per cent. Thrombo-embolism occurred in 29 per cent of these cases. In the non-treated series 30 per cent died. Thrombo-embolic complications developed in 36 per cent of the cases in this series. There was no serious bleeding episode in any of the groups. The results indicate that patients treated with peroral anticoagulants were less likely to develop further thrombo-embolism after the acute coronary occlusion and if they did the chance of dying from it was significantly reduced. Heparin as used in this study did not reduce the incidence of thrombo-embolic complications or the number of deaths resulting from them compared with the non-treated control series. It may be argued that the heparin dosage used in this study was relatively low. However the main objections are the uneven number of cases in the three groups and the deficient information with respect to their comparability.

To our knowledge the only previous study directly concerned with the problem whether an initial course of heparin is a beneficial supplement to peroral anticoagulants in the treatment of patients with acute myocardial infarction, is that of *Eastman et al* (1957). Their material consisted of 362 patients admitted to the same hospital during a period of 7 years. Retrospectively the patients were divided in 3 groups: those receiving no anticoagulant treatment, those receiving Dicumarol and those to whom Dicumarol and an initial course of heparin were administered. The main results are reproduced in Table 1. The untreated group showed the highest mortality rate, but the difference was not of statistical significance unless the deaths occurring during the first 24 hours of admission were included. The group given combined heparin/Dicumarol treatment showed a higher mortality rate than that given peroral anticoagulants only. However the difference was not statistically significant. There was a significantly lower incidence of thrombo-embolism in the anticoagulant treated groups compared with the non-treated series. There was no difference in the incidence of thrombo-embolic complications between the two groups of patients treated with anticoagulants. The results suggest that anticoagulants are of benefit in the treatment of patients with acute myocardial infarction. The increase of hemorrhages, the lack of significant alterations in the mortality rate, and the incidence of thrombo-embolism among cases which received early addition of heparin to peroral anticoagulants, make its use seem unprofitable. The main objection to this study is the unsatisfactory method used for allocation of cases between the groups. The only information on this important point is the laconic statement that the decision concerning the use of anticoagulants rested with the attending physician and that the attitude towards this matter changed slightly from year to year. Thus serious doubt with re-

TABLE 1. *Results abstracted from the study by Eastman et al. The figures in parentheses indicate the results when patients who died within the first 24 hours after admission are included.*

Treatment groups (No. of cases)	Deaths (per cent)	Thrombo-embolism (per cent)	Hemorrhages (per cent)
Untreated 73 (90)	28.8 (42.2)	13.6 (11.1)	1.4 (1.1)
Dicumarol 118 (123)	16.1 (19.5)	3.4 (3.3)	5.9 (5.7)
Heparin & Dicumarol 139 (149)	20.9 (26.2)	3.6 (3.4)	9.4 (8.7)

parin. From the papers reviewed in chapter II it is difficult to assess the separate value of using heparin. In the studies by Glueck *et al.*, Tulloch & Gilchrist, Holten, and Hilden *et al.*, heparin was used routinely in association with peroral anticoagulants until a satisfactory reduction of the prothrombin level was achieved. In the study by Wright *et al* heparin was administered to 15 per cent of the treated group, preferably to those who were critically ill on admission. Feldman added an initial course of heparin to cases where an immediate anticoagulant effect was deemed necessary. Schilling made a similar approach. He was unable to demonstrate any difference in the mortality rate and the incidence of thromboembolism between the group given combined Dicumarol/heparin treatment and that given peroral anticoagulants only. However these groups were not comparable. Most of these studies give deficient information on important aspects of the anticoagulant treatment. This applies especially to the time at which the thrombo-embolic complications occurred and to the anticoagulant effect actually obtained at this moment. Therefore, these studies are without significance in assessing the value of early heparin treatment. One may however suspect that the beneficial effect obtained by anticoagulants in many of these studies might have been attributed to a considerable degree to the initial heparin therapy. This might have been the case in studies where heparin was reserved for patients who were critically ill and in studies where the achieved intensity of the peroral anticoagulant treatment did not tally with the currently favoured requirements. Similar considerations may be applied to the observed incidence of hemorrhagic complications. It is usually impossible to deduce from the various papers whether the bleeding episodes might have been related to heparin or to the peroral anticoagulant regime.

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a) Patients more than 75 years old. This was done to prevent a too heterogeneous age composition of the material. Moreover anticoagulant therapy will often involve practical difficulties in older people.
b) Patients in whom the anticoagulant therapy was delayed more than 72 hours from onset of the acute attack. Late admission to hospital was mainly responsible for this group of patients.
c) Patients experiencing an acute coronary occlusion while already on long-term peroral anticoagulant therapy
d) Patients who died during the first 24 hours after admission. Many of these patients died shortly after admission and were not given anticoagulants at all, or this treatment was only inadequately performed. In any case, maximal effect from anticoagulants can hardly be expected for 24 hours.
e) A small group of patients were excluded for miscellaneous reasons, mainly because of the coexistence of malignant disease

2. Diagnostic criteria

In a study of this kind it is of paramount importance to have well-defined diagnostic criteria. The criteria for the diagnosis of acute myocardial infarction used in this department have been presented in previous papers (Enger *et al.* 1960, Julsrud *et al.* 1961). These criteria were also adhered to during the present study. Patients were included for further study when at least three of the following four features were present:

a) A typical history of anginal pain refractory to nitroglycerine, and/or the presence of acute pulmonary oedema.
b) Electrocardiographic changes, i.e. either changes typical of acute myocardial infarction or signs less characteristic, but altering from one tracing to the next, which were not due to severe extracardiac conditions such as pulmonary infarction. The technique of 12 standard- and unipolar leads was used routinely. Electrocardiograms were recorded daily during the first three days of admission. During the subsequent course tracings were obtained weekly or more frequently when required.
c) Clinical signs compatible with the diagnosis of acute myocardial infarction: fever, leucocytosis (white blood count above 9000 per cmm), elevated and changing erythrocyte sedimentation rate and presence of pericardial friction rub. Clinical signs in accordance with the diagnosis were deemed present when at least two of these four parameters were observed.
d) Elevated serum glutamic-oxalacetic transaminase (GOT) levels. Serum GOT levels were determined daily with a colorimetric method until a normal level was obtained. In accordance with previous experience in this department (Julsrud *et al.* 1961) the upper normal limit was settled at 50 GOT units. With the method employed, 260 GOT units was the maximal value that could be obtained without dilution. For the purpose of this study it was felt unnecessary to make dilutions for the determination of the exact maximal value.

3. The anticoagulant treatment

a) Heparin. The agent used was an aqueous concentrated solution of heparin containing 5000 international units = 50 mg per milliliter (Heparin A. L. pro injectione). To patients admitted on even dates a total daily dosage of 500 mg was administered by 4 intravenous injections equally spaced throughout the 24 hours. If no bleeding episodes occurred this treatment was continued during the first 3 days following admission. No laboratory control was made of the anticoagulant effect of heparin.

b) Phenylindandion. The agent used was ®Trombantin (Nyegaard & Co. A/S), each tablet containing 40 mg. The initial dose of

120 mg was given to both groups of patients as soon as possible after admission to hospital. During the first 1½ years of the study the anticoagulant action of phenylindandion was controlled by the prothrombin and proconvertin (p & p) method of Owren and Aas (1951) while the thrombotest method (Owren 1959) was adopted during the last year. During the first two weeks the prothrombin level was controlled every second day whereas this was done at intervals up to one week during the subsequent course. The level of the desirable anticoagulant effect changed slightly during the study During the first half a reduction of the p & p level between 10 and 30 per cent of normal was accepted as satisfactory However as a consequence of the study by Borchgrevink (1960) a more intensive anticoagulant policy was adopted, the ideal range now being narrowed between 10 and 20 per cent.

c) *Saline* A preliminary statistical evaluation of the results after one year suggested a significantly lower requirement of analgetics in the heparin series. Consequently during the last 1½ years of the study placebo injections were introduced. During the first 3 days of admission patients belonging to the control group were given 4 intravenous saline injections equally spaced throughout the 24 hours. Due to the easily detectable subcutaneous bleedings which regularly occurred at the injection sites of heparin, the double blind technique was not adopted for the heparin/saline injections.

4 Other treatment

Strict bed rest was ordered during the acute phase of the illness, when pain and fever were present. During this period help was given with eating and washing Thereafter the patient was allowed to use a commode and to eat and wash without help. After about three weeks rest in bed the patient was allowed to go to the lavatory and following another week he was permitted out of bed. If no complication had arisen the patient was discharged four to five weeks after admission to hospital.

The medication for cardiac arrhythmias and congestive heart failure followed commonly accepted rules and was uniform in both groups. Patients who presented clinical signs of shock were given pressor agents, preferably nor adrenaline. Pain was treated with morphine or its derivative pethidine.

5 Bleeding episodes and thrombo-embolic complications

Moderate subcutaneous bleedings at the site of the heparin injections were observed in most patients. However only spontaneous subcutaneous bleedings severe enough to cause definite discomfort to the patients were registered as complications and led to discontinuance of the heparin treatment. Epistaxis and all kinds of macroscopic bleedings from the gastro-intestinal and the genito-urinary tract caused immediate stopping of the heparin therapy The peroral anticoagulant treatment was discontinued only if hemorrhagic complications were severe enough to call for blood transfusions or specific agents counteracting the anticoagulant effect.

All patients of the two groups were examined daily with regard to peripheral and systemic thrombo-embolic phenomena. Special attention was paid to patients who experienced periods of irregular fever For the diagnosis of pulmonary thrombo-embolism at least three of the following five features were required. fever acute onset of chest pain with relation to the respiration phase, hemorrhagic sputum, pleural friction rub and pulmonary shadow at the chest X-ray film. If clinical signs of thrombo-embolism developed, the peroral anticoagulant therapy was never supplemented with heparin.

6. Post mortem examination

The autopsy rate at this hospital is fairly high, viz. nearly 90 per cent. The dead patients of this material were submitted to a completely routine post-mortem examination unless this was refused by the relatives. The autopsies were performed by trained pathologists who were unaware of the group to which the patients belonged.

CHAPTER V

Results

1 Description of the material

a) *Some data on the total series of patients studied*

During the period of this study the diagnosis of acute myocardial infarction was made in a total of 575 patients. About 50 per cent of these were excluded according to the criteria given in chapter IV Table 2 shows the distribution of patients among the various causes of exclusion. The *basic* material consisted of 306 patients. Of these, however an additional number of 83 patients or 27 1 per cent, were excluded owing to the occurrence of death within the first 24 hours of admission. Forty-four patients of this group were admitted on even dates and 39 patients on odd dates. Fifty-four of these 83 patients died before the anticoagulant therapy was instituted, whereas this was done in the remaining 29 patients. Of the latter 13 patients belonged to the heparin series and 16 patients to the control series. Thus, there is no difference between the two groups with respect to the number of patients excluded owing to the occurrence of death within the first 24 hours of admission. Finally 4 patients of the heparin series were excluded from some of the calculations because the heparin course had to be terminated owing to the occurrence of a hemorrhagic complication before

TABLE 2. *Survey of the total number of patients with acute myocardial infarction admitted to Ullevål Hospital, Medical Department VII during the period 15 November 1959 to 1 April 1962*

	No. of patients	Per cent
Patients who died before admission to hospital. The diagnosis was established at autopsy (excluded)	26	4.5
Patients experiencing acute myocardial infarction while on long-term anticoagulant therapy (excluded)	43	7.5
Patients in whom the diagnosis of acute myocardial infarction was delayed more than 72 hours from onset of the acute attack (excluded)	59	10 2
Patients above 75 years old (excluded)	132	23 0
Patients excluded because of miscellaneous reasons, mainly the coexistence of malignant disease	9	1.6
Patients constituting the basic material	306	53.2
Total	575	100.0

half of the designated injections had been administered. Thus, the final material comprised 219 patients, of whom 102 belonged to the heparin series and 117 to the control series. These were observed until death or until discharge from hospital. For the surviving patients the mean length of stay in hospital was 32 days in both series.

b) *Comparison of clinical data of the two groups*

It is essential to the interpretation of the results that the two groups should be essentially equal in all important respects except for the anticoagulant regime. Above all this applies to factors known to influence the clinical course of patients with acute myocardial infarction.

The sex distribution is presented in Table 3. There was a relative preponderance of women in the heparin series. However the difference is not statistically significant (chi square value 0.87 i.e. not significant at the 5 per cent level).

The mean age and the percentage distribution according to age groups for the two sexes are presented in Tables 4a, b & c. The two series are shown to be comparable. The well known preponderance of female coronary patients in the older age groups is demonstrated.

Table 5 presents the frequency of some features known to influence the clinical course and the future outlook of patients with an acute coronary occlusion. There was a high incidence of hypertension in the female con-

TABLE 3. *Sex distribution*

	Heparin series		Control series	
	No. of patients	Per cent	No. of patients	Per cent
Males	70	68.6	88	75.2
Females	32	31.4	29	24.8
Total	102	100.0	117	100.0

TABLE 4a. *Age distribution*

	Heparin series		Control series	
	Mean age (years)	Standard deviation (years)	Mean age (years)	Standard deviation (years)
Males	58.7	10.0	58.9	8.5
Females	67.8	6.6	67.2	6.3

TABLE 4b. *Percentage distribution according to age groups (females)*

	Age groups (years)			
	≦45	46-55	56-65	66-75
Heparin series	0	3.1	28.1	68.8
Control series	0	3.4	24.1	72.4

TABLE 4c. *Percentage distribution according to age groups (males)*

	Age groups (years)			
	≦45	46-55	56-65	66-75
Heparin series	10.0	25.7	38.6	25.7
Control series	3.4	31.8	43.2	21.5

TABLE 5. *The frequency of some clinical signs*

		Heparin series	Control series
Reliable information about hypertension (per cent)	Males	11.4	12.5
	Females	18.8	48.3
Clinical signs of shock during the first 48 hours after admission (per cent)		15.7	18.8
Pericardial friction rub (per cent)		20.6	13.7
Cardiac arrhythmia (per cent)		18.6	23.1
Congestive heart failure (per cent)		27.5	25.6

Table 6. *The frequency of previous myocardial infarction and the duration of anginal pain*

		Previous acute coronary occlusion	Anginal pain only related to the present attack of coronary occlusion	Anginal pain of less than one year's duration	Anginal pain of more than one year duration
Heparin series	Males	8.6	54.3	21.4	24.3
(per cent)	Females	15.6	28.1	28.2	43.8
Control series	Males	4.5	45.5	26.2	28.4
(per cent)	Females	13.8	31.0	30.9	37.9

trol patients. When compared with the females of the heparin series the difference in respect of this parameter has a chi square value of 4 75 which is statistically significant at the 5 per cent level. The other parameters are equally distributed between the groups.

Table 6 shows the incidence of previous myocardial infarction and the duration of anginal pain. The difference between the incidence of previous myocardial infarction in the two male groups has a chi square value of 0 68 which is not statistically significant at the 5 per cent level. T ble 6 shows that there is no difference between the groups with respect to the frequency and the duration of anginal pain. More than one year's anginal pain prior to the acute episode of coronary occlusion is a far more frequent finding in female coronary patients than in males. The difference is of statistical significance at the 5 per cent level (chi square value 6.25)

Table 7 shows the percentage distribution of the highest observed serum GOT level of arbitrarily chosen intervals. This parameter is related to the severity of the myocardial damage, and is therefore an indicator of the future outlook. There are only minor deviations in the distribution of the highest serum GOT levels between the groups. The percentage of patients with a maximal GOT value above and below 260 units respectively is exactly the same in the two series.

Patients were included in the study when the anticoagulant therapy was begun within 72 hours from the presumed onset of the acute attack. Table 8 shows that the majority of patients were included in the study during the first 24 hours of the acute illness. There is no difference between the groups with respect to the percentage distribution of patients according to the day of admission from onset of the acute attack.

Table 9 shows the mean serum cholesterol level on admission to hospital. It is evident that the two groups are comparable. For

Table 7 *Percentage distribution of the maximal observed serum GOT level*

	GOT units			
	≤ 40	41 100	101–260	> 260
Heparin series	9 4	40 0	37.7	18.9
Control series	5.1	33.3	42.7	18.8

Table 8. *Percentage distribution of patients according to the number of days from onset of the acute attack to admission at hospital*

Day of admission	Heparin series	Control series
1st	74.5	79.5
2nd	19 6	12.8
3rd	5.9	7 7

TABLE 9 *The mean serum cholesterol concentration on admission to hospital*

	Heparin series		Control series	
	Mean serum cholesterol (mg/100 ml)	Standard deviation (mg/100 ml)	Mean serum cholesterol (mg/100 ml)	Standard deviation (mg/100 ml)
Males	286	69	290	74
Females	319	115	303	54

both sexes the mean cholesterol level exceeds the upper normal limit. The higher mean serum cholesterol level of females compared with males is probably related to the higher mean age of the females.

Table 10 shows that the mean duration of stay in hospital for the surviving patients was the same in both series.

Table 11 shows the results of the roentgenological determination of the relative heart volume (ml per square metre body surface) for the surviving patients. This was done shortly before discharge. There is no difference of statistical significance between the groups. With the employed method the mean heart volume for both sexes is within the upper normal range (Amundsen 1959)

TABLE 10. *The mean duration of stay in hospital of the surviving patients*

	Mean duration of stay in hospital (days)	Standard deviation (days)
Heparin series	32.3	13.1
Control series	32.0	7.5

TABLE 11. *Roentgenological determination of the relative heart volume (surviving patients)*

		Mean heart volume (ml per sq.m body surface)	Standard deviation (ml)
Heparin series	Males	449	82
	Females	448	84
Control series	Males	461	79
	Females	465	139

It seems justifiable to conclude that except for a predominance of hypertensive women in the control series, the two groups are comparable with respect to a great number of important factors known to influence the clinical course and future outlook of patients with acute myocardial infarction.

2. The anticoagulant treatment

The heparin series was given a total of 12 intravenous heparin injections equally spaced throughout the first 72 hours of admission. No laboratory control was made of the hypocoagulability of blood thus obtained.

The peroral anticoagulant treatment was initiated as soon as possible after admission to hospital. It is essential to the comparability of the groups whether the intensity of the peroral anticoagulant treatment was equal in both series. The p & p or the t.t. per cent was determined every second day during the first two weeks of stay in hospital. During the subsequent course this was usually done once or twice weekly. Details of the anticoagulant therapy performed in the fatality cases will be presented later. Figure 1 and Table 12 show the mean p & p or t.t. per cent during the hospital course for the surviving patients. The mean prothrombin level was calculated daily for the first 10 days. For the subsequent course this was done for periods of 5 days. The difference between the mean p & p or

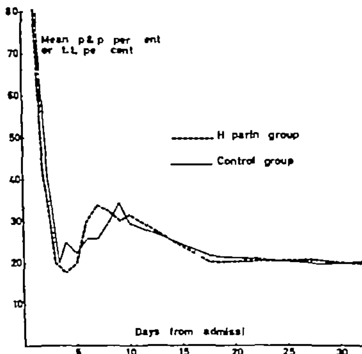


FIGURE 1. *The mean p&p or t.t. per cent of the surviving patients in the two series*

t.t. per cent on the 4th and on the 7th day of admission are of statistical significance at the 5 per cent level. By Student's test the difference on the 4th day has a t value of 2.40 which gives $p < 0.05$. The difference on the 7th day has a t value of 2.59 which also gives $p < 0.05$. When the patients of the heparin series who experienced a hemorrhagic complication are excluded, the difference between the mean values on the 7th day is still of statistical significance ($t = 2.06$ and $p < 0.05$), whereas that on the 4th day is not. The differences between the mean values for the remaining days and periods are not statistically significant. Despite these minor deviations it seems permissible to conclude that the groups are comparable with respect to the intensity of the performed anticoagulant therapy.

The mean dosage of phenylindandion during the first 7 days of admission was calculated in order to decide whether the observed deviations between the prothrombin levels of the series during this period might be related to a difference in the amount of peroral anticoagulants administered. The surviving patients only were included in the calculations. The mean dosage of phenylindandion administered to the heparin series was 421 mg, with a standard deviation of 126 mg. The corresponding value for the control series was 441 mg with a standard deviation of 110 mg. The difference is not statistically significant.

3. *The erroneously grouped patients*

Seventeen patients or nearly 8 per cent of the final material were erroneously grouped and received treatment out of turn. The reason for these failures was mainly related to the method of allocation and will be discussed later. Five patients admitted to hospital on odd dates received heparin, while 12 patients

TABLE 12. *The mean p & p or t.t. per cent of the surviving patients. Only patients in whom the anticoagulant treatment was continued during the entire hospital course are included. The figures in parentheses give the mean prothrombin level for the treated series when those patients are excluded who experienced hemorrhagic complication.*

	Heparin series		Control series	
Day of admission	Mean p & p or t.t. per cent	Standard deviation (per cent)	Mean p & p or t.t. per cent	Standard deviation (per cent)
1	81 (81)	18 (18)	80	21
2	40 (40)	17 (17)	41	17
3	21 (22)	12 (12)	21	11
4	17 (19)	13 (12)	25	14
5	21 (22)	10 (11)	22	10
6	31 (28)	16 (14)	26	13
7	34 (33)	13 (14)	26	12
8	32 (31)	14 (12)	29	10
9	30 (27)	14 (13)	34	16
10	32 (31)	12 (12)	29	14
11–15	27	11	27	11
16–20	20	9	22	9
21–25	21	8	21	9
26–30	21	8	20	9
>30	19	6	20	8

admitted on even dates were wrongly included in the control series. It is important to the interpretation of the results whether these allocation failures influenced the composition of the two groups, i.e. whether they represented a selection of cases. Some data on these patients are presented in Table 13. There was a predominance of females and of patients presenting signs of congestive heart failure among the wrongly grouped cases of the control series. However the difference between the two series with respect to these features was not statistically significant. Nor did the groups deviate from each other or from the total case material in other respects. It may be justifiable to conclude that the wrongly treated patients did not represent any selection of cases. They are therefore included in the total material for further study

4 Mortality figures and some clinical data on the dead patients

a) *The mortality rate*

The mortality figures are presented in Table 14 The mortality rate of the treated series was somewhat higher than that of the control series. The difference, however is far from being of statistical significance; nor was there any difference between the two series when females and males were considered separately The high female mortality rate in the control series was probably related to the high incidence of hypertensive heart dis-

TABLE 13. *Some data on the 17 erroneously grouped patients*

	Heparin series		Control series	
	Males	Females	Males	Females
No. of patients	3		5	7
No. of deaths	1	0	0	1
Thrombo-embolic complications	0	0	0	0
Hemorrhagic complications (No.)	*1*	*2*	*0*	*0*
Previous myocardial infarction (No. of patients)	0		4	
Reliable information about hypertension (No. of patients)	0		1	
Congestive heart failure (No. of patients)	1		5	
Shock during the first 48 hours after admission (No. of patients)	0		1	
Cardiac arrhythmia (No. of patients)	0		2	
Serum GOT above 260 units (No. of patients)	2		2	
Day of admission after the acute onset (No. of patients)	1st: 3 3rd. 2		1st: 12	
Mean duration of stay in hospital for the surviving patients (days)	32		34	

case in this group. However the difference between the mortality rates of the two sexes in the control series was not statistically significant (chi square: 3.25 i.e. $p > 0.05$ but $p < 0.1$)

Previously it has been maintained that the 17 erroneously grouped patients did not represent any selection of cases. However for the sake of completeness, Table 15 shows the mortality figures when these patients are excluded. The original results are uninfluenced by this manipulation.

We also present the total mortality rate of the basic material, i.e. including those who died during the first 24 hours after admission. In these calculations the 17 erroneously grouped patients have been included according to the treatment given. Of the 146 patients admitted on even dates 30.1 per cent died during the first 24 hours after admission while 13.7 per cent died during the

TABLE 14. *Mortality figures*

	Heparin series		Control series	
	Males	Females	Males	Females
No. of patients	70	32	88	29
No. of deaths	14	6	10	8
Mortality rate	20.0	18.8	11.4	27.6
(per cent)	19.6		13.4	

TABLE 15. *Mortality figures when the 17 erroneously grouped patients are excluded*

	Heparin series		Control series	
	Males	Females	Males	Females
No. of patients	67	30	83	22
No. of deaths	13	6	10	7
Mortality rate	19.4	20.0	12.0	31.8
(per cent)	19.6		16.2	

subsequent course. The corresponding figures for the 154 patients admitted on odd dates were 25.3 and 11 7 per cent respectively The total mortality rate of the even date series was 43 8 per cent and of the odd date series 37 0 per cent. The difference is not statistically significant. The over all mortality rate of the basic material was 40 1 per cent.

b) *Some clinical data on the dead patients*

We decided to test whether any disparity existed between the dead patients of the two series with regard to some features known to influence the clinical course of patients with an acute coronary occlusion.

Table 16, a and b shows the mean age and the distribution according to age groups for both sexes. The groups are quite similar

Table 17 presents some clinical features known to influence the outlook of patients with acute coronary occlusion. In the heparin series there is a moderate predominance of patients with serum GOT levels above 260 units. However the difference between the groups with respect to this parameter is not statistically significant. In other respects the groups are quite similar The poor outlook for patients exhibiting clinical signs of shock and of congestive heart failure is demonstrated.

Table 18 a and b shows the period of time elapsing from admission to hospital to the occurrence of death when the fatalities of the first 24 hours were excluded. There was in the heparin series a moderate accumulation of deaths during the first 4 days of ad-

TABLE 16a. *Mean age of the dead patients*

	Heparin series		Control series	
	Males	Females	Males	Females
Mean age (years)	65 6	69.2	61.9	69.0
Standard deviation (years)	10.5	3.6	10.8	5.7

TABLE 17. *Some clinical data on the dead patients*

	Heparin series (No. of patients)		Control series (No. of patients)
Reliable information about hypertension	Males	6	2
	Females	1	4
Serum GOT level above 260 units		11	5
Day of admission after onset of the acute attack	1st	14	14
	2nd	3	4
	3rd	3	0
Previous myocardial infarction		0	1
Shock during the first 48 hours after admission		9	8
Cardiac arrhythmia		4	6
Pericardial friction rub		6	10
Congestive heart failure		16	12

TABLE 16b. *Distribution of the dead patients according to age groups*

	Heparin series (age groups)				Control series (age groups)			
	≦ 45	46-55	56-65	66-75	≦ 45	46-55	56-65	66-75
Males (No. of patients)	1	0	6	7	0	3	3	4
Females (No. of patients)	0	0	1	5	0	0	2	6

mission. During the subsequent 4 weeks the deaths of both series were evenly distributed. The mean length of time from admission to hospital to the occurrence of death was about 12 days for both groups.

Table 19 shows the kind of clinical picture which dominated the final course of the dead patients. The groups are quite similar.

The intensity of the peroral anticoagulant treatment in the two series was compared. Patients surviving the first 3 days of admission were included in this comparison. Table 20 shows that during the final course of the disease the mean p & p or t.t. per cent of the heparin group was lower than that of the control group thus probably indicating a more intensive peroral anticoagulant treatment of the former. The difference however is not of statistical significance. Relatively many patients of both series had a prothrombin level above 25 per cent at the time of death. Quite often, therefore, the peroral anticoagulant treatment performed during the final clinical course deviated from the ideal requirement.

To summarize, there existed no difference between the two series of dead patients with respect to a number of important clinical features.

5. Thrombo-embolic complications

In neither of the two series of patients was there any case of coronary re-infarction during the actual hospital stay.

The diagnostic criteria of pulmonary thrombo-embolism used in this study have been presented above. However the great difficulties in establishing this diagnosis are clearly demonstrated by the fact that one patient of the heparin series and two patients of the control series who fulfilled the criteria,

TABLE 18a. *The mean length of time from admission to the occurrence of death*

	Heparin series	Control series
Mean length of time from admission to death (days)	11.8	12.8
Standard deviation (days)	9.6	8.7

TABLE 18b. *The distribution of deaths according to the length of time from admission to the occurrence of death*

Day after admission	Heparin series (No. of deaths)	Control series (No. of deaths)
1	excluded	
2	2	0
3	3	2
4	3	0
5	0	1
6	0	3
7	1	1
8	0	1
9	2	0
10	0	1
11-15	2	5
16-20	3	0
21-25	1	2
26-30	2	1
>30	1	1

TABLE 19. *The clinical picture dominating the final course of the dead patients*

	Heparin series (No. of patients)	Control series (No. of patients)
Progressive cardio-pulmonary insufficiency	11	7
Sudden death	8	10
Observed ventricular fibrillation	1	1

TABLE 20 *Data on the intensity of the peroral anticoagulant therapy in the dead patients who survived the first 3 days in hospital*

	No. of patients included in the calculations	Mean of the two last (left) and the final (right) p & p or t.t. per cent determinations. Figures in parentheses indicate the standard deviations		No. of patients with a final p & p or t.t. per cent above 25
Heparin series	12	19 (11)	20 (12)	5
Control series	16	27 (16)	27 (14)	7

TABLE 21 *Clinical thrombo-embolic complications*

	Heparin series				Control series			
	No. of patients	Per cent	Deaths (No.)	Deaths (per cent)	No. of patients	Per cent	Deaths (No.)	Deaths (per cent)
Pulmonary thrombo-embolism	1	1.0	0	0	4	3.4	2	1.7
Peripheral thrombo-embolism	0	–	–	–	1	0.9	0	–
Total	1	1.0	0	0	5	4.3	2	1.7

failed to present thrombo-embolism at autopsy performed a few days after the onset of the symptoms and signs suggestive of this condition. In all cases a marked pulmonary congestion was the probable explanation of a clinical picture mistaken for a pulmonary infarction. These patients were therefore excluded from Table 21 which presents the remaining patients in whom this diagnosis was made. One patient only of the heparin series experienced a thrombo-embolic complication.

A 66-year-old man developed clinical signs of pulmonary infarction on the 12th day of admission. On this day as well as two days previously the p & p level was 8 per cent. He made an uneventful recovery.

Five patients of the control series presented clinical signs of thrombo-embolism.

A 45-year-old man developed clinical signs of pulmonary infarction on the 2nd day of admission. The p & p level at this time was 43 per cent. He made an uneventful recovery.

A 46-year-old man developed clinical signs of pulmonary infarction on the 4th day of admission. The p & p level at this time was 15 per cent. The clinical course was uneventful.

A 70-year-old man developed clinical signs of pulmonary infarction on the 9th day of admission. The p & p levels on the 5th and on the 10th day were 17 and 15 per cent respectively. He made an uneventful recovery.

A 74-year-old man developed clinical signs of *peripheral arterial embolism of the lower left extremity* on the 3rd day of admission. The p & p level at this time was 7 per cent. Two and half weeks later an amputation was performed. Four days after the operative procedure he experienced chest pain and clinical signs of respiratory failure, and suddenly died. At autopsy massive thrombo-embolism of the right pulmonary artery with associated infarction of the lung was demonstrated. Additionally there were

left-sided atrial and ventricular mural thrombi. The p & p level fluctuated between 30 and 35 per cent during the last week prior to death.

A 73-year-old woman died suddenly on the 6th day after admission with chest pain and clinical signs of respiratory failure. The day prior to death the p & p value was 27 per cent. Post mortem examination revealed the right pulmonary artery occluded by a large thrombus.

Thus, two deaths in the control series were directly related to a thrombo-embolic complication against none in the heparin series. However this difference is not statistically significant. Neither is the difference between the series in regard to the incidence of thrombo-embolism recognized clinically of statistical significance.

6. Hemorrhagic complications

No hemorrhagic episode was recognized clinically in the control series.

A total of 16 patients* or 15.1 per cent of the heparin series experienced hemorrhagic complications. In all cases except one, the bleeding episode occurred during the first 5 days after admission, i.e. either during the heparin course or during the first 36 hours after the last injection. Seven of these 16 patients had a p & p or t.t. per cent of 10 per cent or lower at the time of the hemorrhage. Spontaneous subcutaneous hemorrhages severe enough to cause discomfort to the patients and bleeding from other sites irrespective of their severity were registered as complications. These caused immediate discontinuance of the heparin treatment. In 11 patients, or 10.4 per cent, the bleeding episodes were mild. These are summarized in Table 22. One patient had a subcutaneous bleeding and died 2 weeks later from congestive heart failure. The remaining patients made an uneventful recovery.

Table 23 presents data on 5 patients of the heparin series (4.7 per cent) in whom the hemorrhagic complication caused a serious clinical situation which called for immediate discontinuance of the heparin treatment as well as of the peroral anticoagulant therapy. To 4 of these patients blood transfusions and vitamin K injections were administered. Two of these patients deserve special attention:

A 6.-year-old woman experienced sudden cerebro-vascular accident with left-sided hemiparesis 36 hours after the last heparin injection. Lumbar puncture revealed sanguinolent spinal fluid showing xantochromia after centrifugation. The day prior to the bleeding the p & p level was 7 per cent. On the day of the event, however, it was 18 per cent. The patient was semi-conscious for some days but made satisfactory recovery except for persisting and disabling hemiparesis.

TABLE 22. *Hemorrhagic complications of minor clinical significance occurring in the heparin series*

Type of bleeding	No. of patients	Therapeutic consequence
Macroscopic hematuria	3	Heparin treatment discontinued. Peroral anticoagulant treatment continued
Epistaxis	1	—»—
Conspicuous subcutaneous hematoma	2	—»—
Conspicuous subcutaneous hematoma	3	Heparin course already completed. Peroral anticoagulant treatment continued
Rectal bleeding	1	—»—
Hematuria	1	—»—
Total	11	

Four patients of the heparin series are included herein who were withdrawn from the previous calculations because heparin had to be omitted due to hemorrhagic complication before less than half of the designated injections had been administered.

3

TABLE 23. *Serious hemorrhagic complications occurring in the heparin series*

Type of bleeding	No. of patients	Therapeutic consequence	Clinical consequence
Hematemesis	1	anticoagulant therapy discontinued	none
Hematuria	1	anticoagulant therapy discontinued. K-vitamin-injections and blood transfusions administered	
Melena	1		none
Intracerebral hemorrhage	1 (see text)	—	hemiparesis
Melena	1 (occurring on the 10th day see text)	—	prolonged period with shock. Death 10 days later
Total	5		

A 61-year-old man of the heparin series who previously had experienced dyspeptic symptoms developed massive gastro-intestinal hemorrhage on the 10th day of admission. The day prior to this event the p & p level was 30 per cent and on the day after the bleeding it was 24 per cent. During the last few days prior to the bleeding he had been given salicylates because of joint complaints. During the first 24 hours after the hemorrhage he was seriously ill with long-lasting period of shock and with complaints of chest pain. There was, however, no electrocardiographic or biochemical sign of progressive myocardial damage associated with this period. The peroral anticoagulant therapy was discontinued. During the first 48 hours he received 3 litres of blood. During the subsequent days he gradually improved; but 10 days after the bleeding episode he suddenly died. The post-mortem examination showed recent thrombotic occlusion of one of the coronary arteries associated with large myocardial necrosis. However, it was impossible to decide whether there had been any progression of the myocardial damage in association with the episode 10 days previously. In addition 3 ventricular ulcers were demonstrated.

Although most of the bleeding episodes occurring in the heparin series were mild, several serious hemorrhages were also encountered. The majority of hemorrhages occurred during or in close connection with the heparin course. However in several of these cases a particular low p & p or t.t. per cent was observed. Therefore, an additional effect of the peroral anticoagulants on the hemostatic mechanisms might have contributed to the occurrence of these hemorrhages. In one case the hemorrhagic complication led to a lasting and disabling condition. In another case of the heparin series the peroral anticoagulant therapy might have contributed to the fatal outcome. However except for this event, the peroral anticoagulant therapy as administered to these groups of patients, has proved to be a safe therapeutic procedure. The difference between the two series of patients with respect to the occurrence of hemorrhagic complications is statistically significant. However this is only the case when the mild and the serious bleeding episodes are treated together.

Table 24 presents the total number of deaths, thrombo-embolism and hemorrhagic complications occurring during the first 6 days of stay in hospital, i.e. at a time when an effect of heparin on the clinical course might be expected. The differences between the series are of statistical significance for hemorrhagic complications only.

TABLE 24. *Deaths, thrombo-embolic phenomena and hemorrhagic complications occurring during the first six days of stay in hospital*

	Deaths (2nd–6th day)		Thrombo-embolic complications		Hemorrhagic complications	
	No.	Per cent	No.	Per cent	No	Per cent
Heparin series	8	7.8	0	–	15 (One intra-cerebral)	14 7
Control series	6	5.1	4 (2 deaths)	3.4 (1 7)	0	–

7 Autopsy findings

In 3 patients of each group the post-mortem examinations were refused by the relatives. Table 25 shows the mean heart weight of the two groups. For both sexes the figures are above the upper normal limit. There is no difference between the two series with respect to this feature. Table 26 shows that an additional number of relevant autopsy findings were equally distributed between the groups. This applies especially to the frequency of recent thrombotic processes within the coronary three, to the incidence of cardiac rupture and to the finding of sanguinolent effusion in the pericardial sac without rupture. In every case the clinical diagnosis of an acute myocardial infarction was confirmed. No attempt was made to evaluate whether retrograde extension of the thrombi within the coronary three subsequent to the acute occlusive process had occurred. In two patients of the heparin series

TABLE 25. *The mean heart weight*

	Heparin series		Control series	
	Males	Females	Males	Females
Mean heart weight (grams)	549	440	525	411
Standard deviation (grams)	86	103	75	80

TABLE 26. *Autopsy findings*

	Heparin series	Control series
No. of autopsies refused by the relatives	3	3
No. of autopsies performed	17	15
No. of patients with an old myocardial infarction not recognized clinically	3	4
No. of patients with atheromatous occlusive processes	10	8
No. of patients with recent thrombotic occlusion of the coronary arteries	9	10
No. of patients in whom no coronary occlusive process was demonstrated	2	2
Rupture of the cardiac wall with hemopericardium	1	1
Serous effusion in the pericardial sac without rupture	2	2
Sero-sanguinolent effusion in the pericardial sac without rupture	2	1
Autopsy findings compatible with chronic congestive heart failure during life	10	7

TABLE 27. *Total number of clinical thrombo-embolisms and thrombo-embolisms disclosed at autopsy*

	Heparin series	Control series
No. of patients	4	6
Per cent	3.9	5.1

there was a marked hemorrhagic necrosis of the myocardial wall, while this was demonstrated in one patient of the control series. Mural cardiac thrombi were found in two cases of the heparin series against one case of the control series. Numerous small emboli of the pulmonary arteries were demonstrated in one case of the heparin series. There was no sign, however of associated pulmonary infarction in this case. Massive thrombo-embolism of the pulmonary artery with infarction of the lung was demonstrated in two cases of the control series. Previously it has been maintained that the clinical course of these cases suggested this complication as the immediate cause of death. Table 27 presents the total number of clinical thrombo-embolism and of thrombo-embolism first disclosed at autopsy the coronary thrombi being excluded. There was no difference between the groups.

8. The consumption of analgetics

Morphine was used as the principal analgetic agent. If the patient suffered from nausea and vomitus, pethidine was preferred. The attending physician made a daily report on the existence of anginal pain and for each patient an exact record was made of the total amount of analgetics parenterally administered during the first 5 days after admission. The mean duration of anginal pain and the mean consumption of analgetics in the two series were compared. Patients who did not experience chest pain after admission were excluded from the statistical treatment.

The control series consisted of two groups of patients, namely those from the first year of the study to whom no placebo was administered and those from the last 1½ years who received *saline by 4 daily intravenous* injections during the first 3 days after admission. Table 28 shows that the percentage of patients who did not experience chest pain after admission was about the same in both series. This is an additional indicator of the comparability of the groups. The mean duration of anginal pain is somewhat shorter in the heparin series than in the control series. The difference, however is far from being of statistical significance ($t = 1.42$) The mean duration of the chest pain in the saline series was 2.9 days (standard deviation 1.4 days) against 2.8 days (standard deviation 1.3 days) in that part of the control group to which placebo was not administered. Thus, there was no difference between the two series of control patients.

Table 29 presents the mean amount of analgetics parenterally administered to the two series. The difference between the mean consumption is of statistical significance at the 5 per cent level ($t = 2.21$ i.e. $p < 0.05$)

There was no difference between the mean

TABLE 28. *The mean duration of chest pain*

	Mean duration of chest pain (days)	Standard deviation (days)	Percentage of patients not experiencing chest pain after admission (per cent)
Heparin series	2.5	1.5	10
Control series	2.8	1.4	12

TABLE 29 *The mean consumption of parenterally administered analgetics during the first five days after admission*

	Mean consumption of analgetics (ml)	Standard deviation (ml)	Percentage of patients to whom analgetics were not parenterally administered after admission
Heparin series	4.3	4.1	26
Control series	5.8	4.7	22

analgetic consumption of the two control groups. The mean consumption of analgetics in the saline series was 5.7 ml (standard deviation 4.4 ml) against 5.9 ml (standard deviation 5.1 ml) in that part of the control series that did not receive placebo. If the mean consumption of analgetics in the heparin group is compared with that of the saline control group only the difference loses its statistical significance at the 5 per cent level, but is close to it ($t = 1.85$ i.e. $p > 0.05$, and $p < 0.1$). This is due to the reduction in the number of control patients when the saline control group alone is taken into account.

These results seem to support the view that heparin may have a favourable influence on the chest pains of patients with an acute coronary occlusion, and that this effect is unrelated to the placebo influence by intravenous injections.

CHAPTER VI

Discussion

The study seems to provide fairly conclusive answers to the questions raised in chapter IV. But some methodological aspects and several of the results obtained are open to discussion.

The criteria for the diagnosis of an acute myocardial infarction used in this study have been discussed in previous papers (Enger *et al* 1960 Jubrud *et al.* 1961). The crucial point is that these criteria were settled prior to the study and that they subsequently were strictly adhered to.

It is an ideal requirement in such a study that the prejudice and the bias of the clinical observer should be avoided by adopting the double blind technique. Originally this was intended. But due to the after-bleedings which regularly occurred at the injection sites of heparin it early became evident that it was impossible to conceal from the observer the anticoagulant regime actually performed. This approach had therefore to be abandoned. Thus, some degree of uncertainty is introduced with respect to the validity of the clinical registration of the thrombo-embolic phenomena. But to the best of our knowledge the patients of both series were submitted to the same daily scrutiny with respect to the existence of thrombo-embolic and hemorrhagic complications.

There was no difference between the groups with regard to the number of patients excluded owing to the occurrence of death within the first 24 hours of admission.

This limit was considered more appropriate than 48 hours, which has been used in many similar studies. With the therapeutic regimes employed a fairly pronounced effect on blood coagulability may be expected in both series from the second day. However the results of the present study are mainly uninfluenced if 48 hours is chosen as the exclusion limit.

The number of patients that remained for final evaluation was not particularly high. But since both groups comprised more than 100 patients the present study is comparable in quantitative respects with most studies designated to evaluate the effect of anticoagulants in myocardial infarction. It may also be questioned whether a therapeutic procedure deserves practical application if more than 200 patients are required to establish its effect. On the other hand the possibility exists that a favourable effect on selected groups of patients remains concealed unless extensive case materials are available for study.

There was a considerable degree of conformity between the groups with respect to a great number of important clinical features. However, there existed one definite difference between the groups, namely a significant predominance of hypertensive women in the control series. We are unable to offer any explanation of this phenomenon, which, however, may merit some practical and theoretical consideration. From the Tables it will be noted that the uneven distribution of hypertensive women remains uninfluenced by regrouping of the erroneously treated patients according to the date of admission. Four of the 8 dead female patients in the control series were hypertensive against 1 out of 6 in the heparin series. Thus, it is conceivable that the high female mortality rate in the control series is related to the accumulation of patients with hypertensive heart disease. In patients with an acute coronary occlusion it is often extremely difficult to decide whether hypertensive heart disease is present or not. This is probably the reason why no statement is made on the incidence of hypertension in many studies of this kind. In the present study patients were accepted as hypertensive if distinctive hypertensive retinal changes were found at ophthalmoscopy or if several blood pressure readings were above 150/100 Hg during the actual hospital stay or had been of that order during previous hospitalization. Serious doubt exists as to the validity of the registration of hypertension in this study. Probably the observed incidence of hypertension represents minimal figures and its significance should be very carefully interpreted. Furthermore, it is important to realize that such a disproportion of hypertensive individuals in the control series will tend to worsen the prognosis.

This skew distribution of a clinical parameter is an interesting demonstration of the fact that important differences might exist between groups of patients selected for clinical trials, even though a fairly large number of patients are included and casual allocation methods used. The importance of testing the two groups of patients obtained with respect to the comparability of all relevant parameters is also emphasized. The important thing is not that such differences exist, but that they are discovered, and that the interpretation of the results obtained is made in accordance with this fact.

This study shows that long-lasting anginal pain prior to the episode of an acute coronary occlusion is a far more frequent finding in female coronary patients than in males. Previously this has been demonstrated in the Framingham study (Kannel *et al.* 1961). The discussion of this interesting phenomenon is outside the scope of this paper.

Although special attention was paid to this possibility 17 patients, or nearly 8 per cent of the total material, were treated out of turn. It has been previously maintained that these failures probably do not represent any selection of cases and that it should be permissible to include them in the calculations according to the treatment actually given. The study was carried out at a large medical department where the great activity and the presence of many doctors increased the chance of misunderstandings. Under such circumstances misgrouping of patients seems to be an almost unavoidable complication in the present method of allocation. A retrospective investigation revealed that the majority of the erroneously grouped patients arrived at hospital around midnight. Misunderstanding related to insufficient information on the exact time of admission to the hospital's reception centre is therefore the probable explanation of the erroneous grouping of many patients. The reason why more patients admitted on even than on odd dates were given treatment out of turn is obscure. The difference is not statistically significant. Previously it has been mentioned that there was moderate preponderance of women in the heparin series. If the erroneously treated patients are redistributed according to the date of admission this disproportion is exaggerated. We are unable to offer any explanation of this phenomenon.

The method of allocation employed has proved to be simple and effective, and has provided two series of patients showing a great degree of conformity But it is an appropriate question whether another approach might have improved the comparability of the groups. By using the more elaborate method of preconstructed random lists, problems related to the misgrouping of patients and to the uneven number of cases between the groups would have been avoided. The latter approach seems therefore to be superior to the alternative date method in clinical studies of this kind. Despite the use of random lists, however two discrepancies between the groups might have persisted, namely the preponderance of females in the heparin series and of hypertensive females in the control group. Ideally when random lists are constructed, adjustments should be made for a great number of clinical factors, among many others the sex distribution and the number of hypertensive individuals in the two series. But this method would only work out satisfactorily if a considerably greater number of patients had been available than in this study

Although the method of randomization employed is open to criticism and undoubtedly might have been improved, it nevertheless may be justifiable to conclude that the present approach has furnished two groups of comparable patients which are suitable for evaluation and statistical treatment. Above all there is no reason to believe that the heparin series contained an undue number of bad-risk cases which might have worsened the outlook for this series and thus concealed a possible benefit from the heparin treatment.

It was demonstrated that the groups were comparable in regard to the intensity of the performed peroral anticoagulant treatment. During the first 3 days of admission there was in both series a pronounced reduction of the prothrombin content of plasma to about 20 per cent of normal. This indicates that the initial phenylindandion dosage of 120 mg on the first day and 80 mg on the second day was appropriate, and furthermore that a 3-day course of heparin was adequate. The conspicuous humps in both tracings between the 5th and 15th day probably reflect a tendency to reduce the phenylindandion dosage more than necessary as consequence of the steep decline of the prothrombin level during the initial phase of therapy An increased resistance to

the anticoagulant agent as a consequence of the improved clinical condition after the acute episode might also have contributed to this phenomenon. The tracings also emphasize the need for frequent blood controls during the initial phase of anticoagulant treatment. Although the general appearance of the two curves is quite similar they deviate from each other during the initial phase. The tracing of the heparin series reaches a lower level and subsequently rises more steeply than that of the control series. The differences between the prothrombin levels on the 4th and on the 7th day after admission are of statistical significance. The amounts of peroral anticoagulants administered to the two series during the first 7 days after admission did not differ significantly from each other The difference obtained on the 4th day probably reflects the sensitivity of the thrombotest method to the influence of heparin (Owren 1959) The steeper rise of the heparin series tracing during the following days compared with that of the control series, is more difficult to explain. Probably it might be interpreted as a rebound phenomenon related to the waning heparin effect. Apart from these minor deviations which we are inclined to characterize as being of no importance the groups are comparable with respect to the crucial point whether the peroral anticoagulant therapy was administered with the same intensity to both series. Although the standard deviations of the prothrombin determinations are fairly great, it seems reasonable to conclude that the anticoagulant effect actually obtained corresponded quite well to that designated. The tracings probably reflect the results which can be expected from an anticoagulant regime when this is administered to a great number of patients in a large medical department.

The mortality figures shown in Table 14 correspond quite well with those of other studies in this field. The mortality rate of the control series is lower than that of the heparin series. The difference is not statistically significant. Nor is there any difference of statistical significance between the groups when the sexes are considered separately The difference between the mortality rates of females and males in the control series is nearly of statistical significance. Previously it has been maintained that the high female mortality rate in the control series is probably related to the predominance of hypertensive individuals in this group Since there is no reason to believe that patients admitted on even dates represent a selection of cases with a more unfavourable outlook than those admitted on odd dates, the mortality figures of the present study give no support to the assumption that heparin might exert a favourable influence on the immediate prognosis of patients with acute myocardial infarction.

With regard to a number of clinical features there was no difference between the dead patients of the two groups. The mean length of time from admission to the occurrence of death was the same in the two groups. In the heparin series there was a moderate accumulation of deaths occurring during the first days of admission. However the difference between the groups was not of statistical significance. After excluding the fatality cases of the first 24 hours, the deaths of both series were evenly distributed throughout the first 4 weeks following the acute coronary occlusion. The anticoagulant regime was performed with less intensity in the series of dead patients than in those who survived. Probably this is due to difficulties attendant on the administration of this therapy to patients severely ill. The effect obtained by the peroral anticoagulants in the dead patients of the heparin series was of greater intensity than in the control series. But the difference is not of statistical significance It is important, however that the

dead patients of the heparin series did not receive a less adequate anticoagulant regime than those of the control series. If this had been the case, the potential benefit of heparin might have been counterbalanced.

The autopsy findings of the two series do not deviate from each other in any important respects. In every case the clinical diagnosis of acute myocardial infarction was confirmed. The most prominent feature was the low incidence in both series of mural intracardiac thrombi. In about two-thirds of the cases in both series a recent thrombotic process was demonstrated. In two cases of each group no definite occlusive process was found. In the remaining cases the coronary arteries showed extensive atheromatous changes which might well have effected a total occlusion during life. No attempt was made to evaluate whether there had been a retrograde extension of the thrombus within the coronary three. With conventional methods this is probably an insurmountable task. The two series showed the same low incidence of rupture of the cardiac wall and of sero-sanguinolent effusion into the pericardial sac without rupture. Two cases in the heparin series showed a marked hemorrhagic necrosis of the cardiac wall as against one case in the control series. It may therefore be concluded that with respect to a great number of relevant features disclosed at autopsy the two groups did not differ significantly from each other

Compared with most of the studies reviewed previously an exceptionally low incidence of thrombo-embolic complications was demonstrated. To the best of our knowledge no case was missed, and we are inclined to presume that the observed incidence of thrombo-embolism represents maximal rather than minimal figures. Thus, three cases fulfilling the clinical criteria of a pulmonary infarction had to be excluded owing to the fact that post-mortem examinations performed a few days later revealed marked pulmonary congestion only In the heparin series there was only one case of pulmonary thrombo-embolism. This occurred on the 12th day after admission. In 4 cases of the control series, two of which were fatal, the symptoms and signs of thrombo-embolism occurred within the first 6 days after admission, i.e. at a time during which heparin, if administered, might have influenced the coagulability of blood. The thrombo-embolic phenomena occurred in 4 cases at a time when an appropriate reduction of the prothrombin level was achieved, i.e. at a p&p or t.t. per cent ranging from 8 to 17 This is an illustration of the well-known clinical experience that a thrombotic process might develop despite the reduction of the prothombin content of plasma to a desirable anticoagulant level. However a considerable length of time may elapse from the initiating of a thrombotic process until its clinical manifestation. Therefore the exact timing of a thrombotic process in relation to the actual prothrombin level is a difficult task. No definite importance can be attached to the fact that in both cases with a fatal pulmonary thrombo-embolism the process developed at a time when the prothrombin level was above 30 per cent. One can, however speculate whether these fatalities might have been prevented by an optimal anticoagulant regime. The moderate difference between the series in regard to the incidence of thrombo-embolism and of the mortality rate from this complication is far from being of statistical significance. The difference between the series with respect to thrombo-embolic phenomena recognized clinically is almost counterbalanced when those at first disclosed by post-mortem examinations are added.

Hemorrhagic complications were encountered in total of 16 patients, all of

whom belonged to the heparin series. The majority of the bleeding episodes were mild. In all cases except one the complication was related to the heparin treatment. But in nearly half of these cases the prothrombin level was reduced to 10 per cent of normal or less at the time of the hemorrhage. It has been maintained above that heparin can influence the results of the thrombotest method. Thus it is impossible to decide to what extent an additional effect on the blood coagulability by phenylindandion might have contributed to the hemorrhagic complications. In the studies previously cited the incidence of bleeding complications associated with anticoagulant therapy in acute myocardial infarction varies between 2 and 20 per cent. In most of these studies it is difficult to differentiate between the bleedings due to the heparin treatment and those related to the peroral anticoagulant therapy. In the present series of patients, however, only one of the hemorrhagic episodes was clearly related to the phenylindandion therapy. This complication, however, was a serious one which probably contributed to the fatal outcome. On the other hand, this patient was by mistake given salicylates prior to the hemorrhage. In the control series there was no bleeding episode recognized clinically. But in one of the fatal cases of this series the casual finding of a sero-sanguinolent pericardial effusion was made at autopsy. Retrospectively it was felt impossible to evaluate whether this complication had contributed to the fatal outcome. It seems permissible to conclude that despite the achievement of a relatively high intensity of the peroral anticoagulant regime, in the present study this has proved to be an essentially safe therapeutic procedure. When the moderate and serious hemorrhagic complications of the heparin series are treated together, there is a difference of statistical significance between the groups with respect to this feature. It may be argued that a relatively high heparin dosage was employed. When, however, the heparin dosage was established, it was felt essential to anticipate later argument that a possible poor effect of heparin in this study might be related to an insufficient dosage scheme.

In the great Copenhagen study the rate of hemorrhagic complications encountered was 19 per cent, i.e. of the same order of magnitude as in the heparin series of the present study. In the Danish paper there is no information on the time at which the hemorrhages occurred. It is therefore impossible to decide whether the great number of bleedings in this study was related to heparin or to the peroral anticoagulant therapy. In the present study, however, there seems to be ample evidence for the assumption that the majority of the bleedings in the Danish study might also have been related to the heparin treatment. Due to the presumed disproportion between the benefit and the danger of anticoagulant therapy this regime has been omitted in the treatment of patients with acute myocardial infarction in many Danish hospitals. The results of the present study would probably justify a re-evaluation of this decision.

From the present study it seems justifiable to conclude that heparin is without benefit in the management of patients with acute myocardial infarction. It is appropriate, however, to raise the question whether there exist reservations to this statement. For instance, do the results suggest any tendency which might have become manifest in a study comprising a greater number of cases? Although of no statistical significance, in the control series there is a moderate accumulation of thrombo-embolic phenomena observed clinically. This tendency, however, is almost counterbalanced when the thrombo-embolic complications disclosed at autopsy are added. Among those experiencing a thrombo-embolic episode, the only deaths from

pulmonary infarction occurred in the two oldest individuals. Furthermore the difference between two fatalities and none in case materials of this magnitude is very far from statistically significant. Therefore it seems permissible to conclude that no evidence can be presented in favour of the assumption that the inclusion of a greater number of patients might have influenced the results obtained. Another question which deserves attention is whether a favourable effect of heparin on selected groups of patients only might have been concealed in the presentation of the over all results. The subgroups of the present material are too small to provide the complete answer to this question. However from the Tables and the details presented on the dead patients and on those with thrombo-embolic complications, there is nothing to suggest that heparin might exert a favourable effect on subgroups of patients when the sexes and the different age groups are considered separately

It is also an appropriate question whether heparin might have exerted an *unfavourable* effect on the clinical course apart from the hemorrhagic complications obviously present. Previously it has been maintained that the high female mortality rate of the control series is related to the predominance of hypertensive individuals in this group. But in the considerably larger series of male patients there is a higher mortality rate in the heparin series than in the control series. The difference, however is not statistically significant. One should also bear in mind the preponderance in the heparin series of deaths occurring from the 2nd to the 5th day after admission. But the difference between the groups is not statistically significant. Finally the post-mortem examinations failed to reveal any evidence for the assumption that heparin might have contributed to the fatal outcome by producing hemorrhages in the damaged heart muscle. Although the present study does not permit definite conclusions, it seems reasonable to maintain that apart from the hemorrhagic complications obviously present, there is no reason to believe that heparin might have exerted an unfavourable influence on the clinical course of these patients.

Since heparin is without any influence on the clinical course of patients with acute myocardial infarction it may be permissible to combine the two groups in this study and to evaluate the total incidence of thrombo-embolic complications occurring in this series of 219 patients. By this manipulation the rate of fatal thrombo-embolism is found to be 0.9 per cent, the rate of thrombo-embolic complications recognized clinically is 2.7 per cent and the combined rate of thrombo-embolism observed prior to death and at autopsy is 4 6 per cent. Of course these data do not permit general conclusions as to the value of peroral anticoagulants in the treatment of patients with acute myocardial infarction. Above all, early mobilization may have contributed to these results. To the authors knowledge however these figures are more favourable than those so far reported in the literature from studies comprising treated or non-treated case materials with a comparable age and sex composition. Furthermore, the incidence in the present study of hemorrhagic complications clearly related to the peroral anticoagulant therapy was unusually low namely less than one per cent. This indicates that with the employed method of blood control the use of phenylindandion is a safe therapeutic procedure in the treatment of patients with acute myocardial infarction.

During the first 5 days of admission a significantly smaller amount of analgetics was administered parenterally to the patients of the heparin series compared with the control series. Placebo injections were used

during the last two-thirds of the study only. When the mean analgetic consumption of the control patients from this period is compared with that of the heparin series, the difference loses its statistical significance (p greater than 0.05 and less than 0.1). This is due to the smaller number of cases now constituting the control series. Nevertheless, the results reveal a definite trend which seems to justify the conclusion that heparin may exert a favourable influence on the chest pains of patients with acute myocardial infarction. It is difficult to offer a clear-cut explanation of this phenomenon. It has been demonstrated in man that the rapid clearing of plasma lipemia by heparin is associated with a significant increase in tissue oxygen tension (Joyner *et al.* 1960). This increment is unrelated to changes in blood flow and might be attributed to an augmented diffusion of oxygen from (or to) the erythrocytes. The observed analgetic effect of heparin on the chest pains in this series of patients may therefore be ascribed to an increased myocardial oxygen tension related to the plasma-clearing effect of heparin. However, in the present study this potential increment of the myocardial oxygen tension was without any influence on the clinical course.

Chapter VII

Summary

In *Chapter I* the commonly accepted rules for the designing and the performance of controlled clinical trials are reviewed and discussed with special reference to the evaluation of the benefit of anticoagulants in the treatment of patients with acute myocardial infarction.

In *Chapter II* some well-known studies on this subject are reviewed and discussed. Few of these comply with the requirements established in *chapter I*. Evaluation of various studies from different parts of the world seems to justify the conclusion that the use of anticoagulants is of some slight but definite benefit in the management of patients with acute myocardial infarction. This benefit is manifested by a reduction of thrombo-embolic phenomena and of deaths from this complication. In most studies the use of peroral anticoagulants has been combined with heparin therapy during the initial clinical course. From these studies it is impossible to evaluate the relative importance of the two different anticoagulant regimes. The same applies to the hemorrhagic complications due to this combined therapy.

In *Chapter III* a review is presented of previous studies designated to evaluate the benefit of peroral anticoagulants only versus this treatment associated with an *initial* heparin course in the treatment of patients with an acute myocardial infarction. None of these studies fulfils the requirements of a controlled clinical trial.

In *Chapter IV* the purpose and the approach of the present investigation are outlined. The study was designed to evaluate whether in-

tal heparin treatment might represent a useful supplement to peroral anticoagulants in the treatment of patients with acute myocardial infarction. Two groups of patients were studied with respect to the mortality rate and to the incidence of thrombo-embolic and hemorrhagic complications observed clinically or at autopsy. The patients were observed until discharge from hospital. The material was also used to test the hypothesis that heparin might have a favourable influence on the chest pains in patients with acute myocardial infarction. Patients admitted on even dates were given a 3-day course of heparin consisting of a daily dosage of 500 mg administered by 4 intravenous injections equally spaced throughout the 24 hours. A preliminary statistical evaluation of the results after one year suggested a significantly lower requirement of analgetics in the heparin series. Consequently during the last 1½ years of the study a 3-day course of 4 daily intravenous placebo injections with saline were administered to patients admitted on odd-numbered days. No control was made of the hypocoagulability of blood induced by heparin. To both series phenylindandion was administered in a dosage sufficient to reduce the prothrombin content of plasma to about 20 per cent of normal as controlled by the prothrombin/proconvertin or the thrombotest method. Originally it was intended to follow the double blind method. This approach had to be abandoned due to the distinct after-bleedings which regularly occurred at the injection sites of heparin. At autopsy the pathologist was unaware of the group to which the patients belonged. To increase the homogeneity of the material the following groups of patients were excluded: patients more than 75 years old, patients already on long-term peroral anticoagulant therapy, patients with an acute myocardial infarction supposed to be more than 3 days old and patients who died within the first 24 hours after admission.

In *Chapter I* the case material and the results are presented. During the 2½-year period of the study a total of 575 patients were observed. Thirty-eight per cent or 219 patients were selected for final evaluation. Twenty-seven per cent of the patients were excluded owing to the occurrence of death within the first 24 hours after admission. These deaths were evenly distributed between the two series. The remainder were excluded for the reasons mentioned above. The heparin series consisted of 102 patients and the control series of 117 patients. These were observed during the acute illness, i.e. until death or until discharge from hospital. The series were found comparable with respect to a large number of important clinical features: the age and sex composition, the occurrence of previous myocardial infarction, the percentage distribution within the groups of serum GOT levels, the incidence of shock, cardiac arrhythmia, congestive heart failure, pericardial friction rub, the time of admission to hospital, the intensity of the performed peroral anticoagulant regime and the duration of stay in hospital. In one respect only did the groups deviate from each other. There was a significant preponderance of hypertensive females in the control series.

Seventeen patients were given treatment out of turn. These failures were mainly due to misinterpretation of the exact hour of admission to hospital. No selection was involved, and these patients were included in the study according to the treatment given.

The mortality rate of the heparin series was 19.6 per cent and of the control group 15.4 per cent. The difference was not statistically significant. Neither was there any difference between the groups when the sexes and different age groups were considered separately. The results remained uninfluenced when the erroneously grouped patients were excluded. In the heparin series there was a slight accumulation, without statistical significance, of deaths occurring

from the 2nd to the 5th day after admission. Apart from this, the deaths were evenly distributed throughout the first 4 weeks, the fatality cases of the first 24 hours being excluded. The post-mortem examinations failed to reveal any difference between the groups with regard to: the heart weight, the demonstration of a recent coronary thrombotic process, the existence of extensive atheromatous lesions, the incidence of hemorrhagic necrosis of the myocardium, the incidence of mural thrombi within the cardiac chambers, cardiac rupture, and the existence of sanguinolent pericardial effusion without rupture.

In these series there was no occurrence of a definite coronary reinfarction or the development of a post-infarction syndrome. In the heparin series there was only one case of pulmonary infarction (1.0 per cent). This complication developed on the 12th day and the patient made an uneventful recovery. In the control series there were 5 thrombo-embolic complications (4.3 per cent). Four of these occurred during the first 6 days of the hospital course. Four patients developed a pulmonary infarction and the two oldest of these died. In the control series the mortality rate from thrombo-embolic complications was 1.7 per cent. The difference between the series with respect to the incidence of thrombo-embolism and to the mortality rate from this complication is not statistically significant. If the number of thrombo-embolic phenomena first disclosed by post-mortem examination is added to those recognized clinically, the total rate of thrombo-embolism in the heparin series is 3.9 per cent against 5.1 per cent in the control series. This difference is not statistically significant.

In the control series no hemorrhagic complication was recognized clinically. In one of the fatality cases of this series a sero-sanguinolent pericardial effusion without rupture was demonstrated.

Eleven patients (10.4 per cent) in the heparin series experienced a hemorrhagic complication of minor clinical significance. In all cases the episode was probably related to the heparin treatment. Five patients (4.7 per cent) in the heparin series experienced a serious hemorrhagic complication. Four of these episodes were probably related to the heparin therapy. In one case an intracerebral hemorrhage developed with a persisting and disabling hemiparesis. In one case in the heparin series a serious gastro-intestinal hemorrhage developed on the 12th day after admission. Probably this event contributed to his death 10 days later. By mistake the patient had been given salicylates prior to the hemorrhage. When the serious and the moderate hemorrhagic complications of the heparin series are added, the difference between the groups with respect to this complication was of statistical significance.

During the first 5 days of admission a significantly smaller amount of analgetics was administered parenterally to the heparin series compared with the control series. Placebo injections were administered to the control series during the last two-thirds of the study. If the consumption of analgetics of these patients only is compared with that of the heparin series, the difference loses its statistical significance (p between 0.05 and 0.1). The results do, however, reveal a definite trend which seems to justify the conclusion that heparin may exert a favourable influence on the chest pains in patients with acute myocardial infarction.

In *Chapter VI* the method of study and the results obtained are discussed. The allocating method of the present study provided two series of comparable patients suitable for evaluation according to the actual purpose. There are minor deviations, however, between the groups. This applies to the uneven number of patients in the two groups, to a

slight accumulation of females in the heparin series and to a definite preponderance of hypertensive females in the control series. These differences as well as the misgrouping of patients could have been prevented by using preconstructed random lists, if necessary supplemented with adjustments for important clinical features. In clinical studies of this kind the use of random lists seems to be superior to the alternate date method.

The results obtained furnish no evidence for the assumption that the inclusion of a greater number of patients might have altered the outcome. Nor was there any reason to believe that heparin had a favourable influence on the clinical course of selected groups of patients. Conversely there was no evidence for the assumption that heparin might have an unfavourable influence on the clinical course of patients with acute myocardial infarction apart from the hemorrhagic complications obviously present. If these two series comprising 219 patients are treated together the rate of thrombo-embolism recognized clinically was 2.7 per cent and the mortality rate from this complication was 0.9 per cent. Taking the age and the sex composition of this material into account,these figures are exceptionally low There was also an unusually low incidence of hemorrhagic complications clearly related to the peroral anticoagulant therapy (less than 1 per cent) Thus, with the employed method of blood control the use of phenylindandion has proved to be a safe therapeutic procedure in the treatment of patients with acute myocardial infarction.

It is suggested that the analgetic effect of heparin demonstrated in this series of patients was the consequence of increased oxygen tension of the cardiac muscle related to the plasma-clearing effect of heparin.

Conclusions

1 In patients with acute myocardial infarction treated with peroral anticoagulants, initial heparin therapy is without effect in preventing thrombo-embolism and death from this complication.
2. With the dosage scheme employed, heparin significantly increases the risk of hemorrhagic complications.
3 Heparin may diminish the chest pains of patients with acute myocardial infarction.

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VON

EERO VARTIAINEN

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DIE FETALEN ERYTHROZYTEN

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 398

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VORST.: D. PROF. DR. med. AARNO TURUNEN

DIE FETALEN ERYTHROZYTEN

EINE UNTERSUCHUNG ÜBER DIE IM LAUF DER FETALENTWICKLUNG AUFTRETENDEN WANDLUNGEN DER FÄRBBARKEIT UND FREQUENZ DER ROTEN BLUTZELLEN, DIE AUF QUALITATIVEN UND QUANTITATIVEN ÄNDERUNGEN DES HÄMOGLOBINS BERUHEN

VON

EERO VARTIAINEN

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INHALTSVERZEICHNIS

EINLEITUNG

Die von Körber 1866 gemachte Beobachtung, dass das Nabelschnurblut gegen Alkalidenaturation resistenter ist als das Erwachsenenblut, wurde erst i. J. 1910 von Wakulenko endgültig bewiesen.

Danach ist die Differenzierung des Erwachsenenhämoglobins vom Fetalhämoglobin physikalisch, chemisch und serologisch nachgewiesen worden.

Fussend auf dem Unterschied in der Alkalidenaturation dieser beiden Hämoglobine hat man zahlreiche Methoden entwickelt mit deren Hilfe das fetale Hämoglobin qualitativ und quantitativ im Lauf der Fetalentwicklung und auch noch nach der Geburt bestimmt werden konnte. Alle diese Verfahren sind jedoch mit dem Nachteil behaftet dass man mit ihrer Hilfe nur Hämolysat untersuchen kann wobei die eventuellen durch Verschiedenheit des Hämoglobins verursachten, spezifischen Eigenschaften der fetalen Erythrozyten nicht erfasst werden. Diesen Nachteil haben Kleihauer Braun Betke (1957) behoben indem sie eine und Färbemethode entwickelten die auf der verschiedenartigen Löslichkeit dieser beiden Hamoglobine in saurem Puffer mit bestimmtem pH beruht die Zellstruktur intakt lässt und so die Möglichkeit bietet die fetalen Erythrozyten einzeln im gefarbten Präparat zu betrachten.

Da man annehmen darf dass in der Symbiose Fetus-Plazenta Mutter gegenseitige Transfusion stattfinden kann ist dieses Färbeverfahren bei dem die Zellstruktur intakt bleibt natürlich auch zur Untersuchung dieser Frage angewandt worden.

Zahlreiche Autoren sind auch zu dem Resultat gekommen, dass in der Schwangerschaft und zwar besonders im Endstadium, sehr oft ohne wahrnehmbaren Grund fetalhämoglobinhaltige Erythrozyten im peripheren Kreislauf der Mutter auftreten. Bei gewissen obstetrischen Komplikationen sind Frequenzzahlen der Fetalerythrozyten festgestellt worden die deutlich über dem gewöhnlichen Niveau liegen (Zilliacus u. Mitarb 1961 Oehlert 1963) Insbesondere in den Fallen, wo in der Plazenta pathologisch vermehrte Blutströmung vom Fetus zur Mutter vor sich geht ist es wichtig zu unterscheiden, welche Erythrozyten vom Fetus herstammen. Dies kann unschwer dann geschehen wenn man mit Bestimmtheit weiss dass alle die Zellen die sich in der mütterlichen Blutprobe nach der Elution anfärben tatsach-

lich rote Blutkörperchen des Fetus sind. Dies ist auch meistens der Fall aber die Sache wird dadurch kompliziert dass bei manchen hämatologischen Krankheiten auch beim Erwachsenen Fetalhämoglobin produziert wird.

Abgesehen davon, dass die fetalen Erythrozyten entweder fetales oder sog bleibendes Hämoglobin enthalten können ist es nach Kleihauer und Betke (1962) auch möglich dass beide Hämoglobine nebeneinander in ein und derselben roten Blutzelle vorkommen. Da ferner die Beobachtung gemacht worden ist dass ausser diesen beiden Hämoglobinen nach ein drittes für das Anfangsstadium der Fetalentwicklung charakteristisches primitives Hämoglobin auftritt darf man mit gutem Grund annehmen dass man durch Färbung von fetalen Blutproben Erythrozytentypen unterscheiden könnte die in verschiedener Weise auf die Färbung reagieren Die Identifizierung dieser Zellen und die Verfolgung ihrer Frequenzverhältnisse im Lauf der Fetalentwicklung könnten uns neuen Einblick in die Hämatopoese gestatten und neue Möglichkeiten auch für die klinische Anwendung eröffnen.

DIE HÄMATOPOESE

Die fetale Hämatopoese

Die Blutbildung der Fetalzeit wird gewohnterweise in drei Hauptabschnitte eingeteilt, je nachdem wo die Bildung der Blutzellen vor sich geht. In der ersten sog *mesoblastischen Periode* entwickeln sich die Zellen hauptsächlich im Dottersack. Bereits in der zweiten Embryonalwoche bilden sich Zellverdichtungen *Blutinseln* heraus in denen die Mesenchymzellen sich abplatten und rings um diese Inseln herum legen wo sie die Anlage einer Gefässwand bilden während wieder die im Innern zurückbleibenden Zellen Vorstufen der Blutzellen sind Diese mesoblastischen Zellen sind polyvalent und können offenbar als eine Art unspezifischer Vorstufen der Blutzellenbildung angesehen werden. Die ersten schon so weitgehend differenzierten Zellen dass man sie als Blutzellen bezeichnen kann, und die erwiesenermassen Haemoglobin enthalten sind die sog *primitiven Erythroblasten* Diese Zellen gehören noch in die mesoblastische Periode. Erst im zweiten Fetalmonat setzt Blutzellenbildung auch in der Leber ein die dann mit geringer Unterstutzung der Milz im Laufe der beiden folgenden Fetalmonate in der Hauptsache für die ganze Hamatopoese sorgt Wir sind damit zur *hepatolienalen* Periode gekommen

Ausgangs des dritten Fetalmonats dürfte die mesoblastische Blutzellenbildung schon so gut wie abgeschlossen sein und erst Anfang des fünften Monats beginnt die Blutbildung in einem ganz neuen Gebiet, nämlich im Knochenmark. Damit setzt die dritte sog *medulläre Periode* ein Etwa im gleichen Verhältnis wie der Anteil des Knochenmarks an der Blutbildung zunimmt, geht die Beteiligung des hepato-lienalen Systems zurück, und zur Zeit der Geburt sorgt das Knochenmark praktisch genommen allein für die gesamte Hämatopoese

Diese Einteilung in drei Gruppen ist natürlich keineswegs ganz exakt, sondern sie liefert nur den schematischen Rahmen fur den vielseitigen Prozess der die fetale Hämatopoese einschliesst. Unterzieht man diese Einteilung näherer Betrachtung, so sieht man sofort, dass die erste Periode ganz wesentlich von den beiden folgenden insofern abweicht, als die Blutzellenbildung an kein bestimmtes Organ gebunden sondern ein diffuser Vorgang im Mesenchym ist. Logischerweise kann man dann auch eine abweichende Beschaffenheit der in dieser Periode produzierten Zellen erwarten. Diese Zellen

der ersten Gruppe besitzen denn auch fast alle einen Kern und sind deutlich grösser als die Zellen der späteren Generationen Aufgrund dessen ist eine Einteilung in zwei Perioden vorgeschlagen worden nämlich die *megaloblastische* und die *normoblastische* wonach diese grossen plumpen kernhaltigen Zellen Megaloblasten und die kleineren grösstenteils kernlosen Zellen Normoblasten wären

Knoll (1931) und Mundorff (1927) haben in ihren morphologischen Untersuchungen nachgewiesen dass die Megaloblasten in den drei ersten Fetalmonaten deutlich die Mehrheit ausmachen während wieder in den zwei letzten Dritteln die Normoblasten überwiegen Bezüglich der Frage ob Megaloblasten und Normoblasten genetisch verschieden sind oder ob der Unterschied nur in der Karyokinese liegt sind ganz entgegengesetzte Ansichten vorgebracht worden Knoll und Naegeli (1900) vertreten den Standpunkt dass ein wirklicher Unterschied in der Genetik besteht aber die jüngeren Forschungen haben zu der Vermutung Anlass gegeben dass der Unterschied doch in erster Linie karyokinetisch sei Diese Auffassung wird am deutlichsten von den Untersuchungen gestützt die in Fällen von megaloblastischer Anämie durchgeführt worden sind (Davidson Davis & Innes 1942 sowie Zuelzer & Ogden 19 0)

Wie es sich nun auch mit den Grundlagen dieses Unterschiedes verhalten mag, ist auf jeden Fall nachgewiesen worden dass die Megaloblasten die im ersten Drittel der Fetalzeit die Mehrheit ausmachen eine Art Stammzellen der normoblastischen Erythrozyten sind die vielleicht unter der Einwirkung von Biokatalysatoren welche den Kernstoffwechsel beeinflussen über mehrere Generationen zu Normoblasten werden

Die postnatale Hämatopoese

Über die Frage welcher Zellentyp als Grundzelle der postnatalen Hämatopoese anzusehen wäre sind viele einander völlig widersprechende Auffassungen vorgebracht worden Nach der sog *unitaren Theorie* soll es eine polyblastische Zelle geben welche die Stammform der ganzen hämatopoetischen Zellenbildung wäre. Der namhafteste Vertreter dieses Standpunktes ist Maximow (1927) während wiederum eine andere bekannte Autorität Ehrlich (1891) für die dualistische Theorie eintritt. Nach dieser letzteren gibt es zwei Stammzellen die myeloblastische und die lymphoblastische Schilling (1911) fügt noch eine Stammzelle der Monozyten hinzu und so kommt die *trialistische* Theorie zustande. Heutzutage herrscht jedoch fast ausschliesslich die Auffassung, dass jede Zellenart ihre eigene Stammzelle besitzt die sich über genau bestimmte Entwicklungsstadien zur reifen Blutzelle heranbildet. Freilich halten auch heute noch manche Forscher daran fest dass im Knochenmark normalerweise pluripotente Zellen vorkämen (Bessis 1948 Ferrata & de Negreiros 1914) Hiergegen spricht jedoch die Tatsache dass diese undifferenzierten offenbar dem megaloblastischen Typus zugehörigen postnatal nur bei schweren patho-

logischen Zuständen wie z.B bei per niziöser Anämie auftreten.

Was also für die Blutbildung in der Fetalzeit gilt lässt sich nicht ohne weiteres auf die postnatalen Verhältnisse anwenden schon aus dem einfachen Grund dass in der megaloblastischen Periode *die Blutbildung ubiquitär* im Mesenchym stattfindet während *sie gegen Ende der Fetalzeit und na* türlich nach der Geburt an die blutbildenden Organe gebunden ist. Weickers (1954 1957) karyometrischen Untersuchungen haben auch gezeigt dass im Knochenmark postnatal als Stammzelle der Blutbildung eine klar differenzierte Zelle auftritt die in der Erythropoese als Proerythroblast bezeichnet wird

Die fetale Erythropoese

Wenn wir von der Gruppierung ausgehen welche die fetale Hamatopoese in eine megaloblastische und eine normoblastische Periode einteilt können wir leicht verstehen dass die fetale und die postnatale Blutbildung als zwei verschiedene Erscheinungen aufzufassen sind

Im Lauf der zwei ersten Fetalmonate geht die Blutzellenbildung in der Hauptsache im embryonalen Mesenchym vor *sich aber schon Mitte* des zweiten Monats setzt auch die zum *normoblastischen System gehörige in* erster Linie in der Leber stattfindende Zellenbildung ein Diese megaloblastischen Zellen sind gross haben einen Durchmesser bis zu 25 μ und sind im reifen Zustand hyperchromatisch. Anfänglich ist auch der Kern gross und weist ein feines Chromatinnetz sowie Nukleolen auf Nach und nach werden die Kerne etwas *kleiner* bleiben aber doch im grössten Teil der Zellen bestehen Nur 10—15 / von den Zellen dieser Periode sind kernlos und diese Zellen bezeichnet Mundorff als *Megalozyten* Diese gewissermassen primitiven *Zellen vermehren sich* wie *beobachtet* worden ist sowohl durch Mitose *Promitose wie auch Amitose* (Maximow Knoll) Spektroskopisch ist nachgewiesen worden dass der Farbstoff der Megaloblasten Hämoglobin ist.

In der normoblastischen Periode ist die Blutzellenbildung an das Mesenchym bestimmter blutbildender Organe gebunden. Anfänglich erscheinen in der Leber grosse kernhaltige Zellen aus denen dann die ersten kernhaltigen Normoblasten hervorgehen. Selbst die grössten von diesen Zellen sind nicht so gross wie die megaloblastischen Urzellen. Die Vermehrung der normoblastischen Zellen läuft nach den gleichen Prinzipien ab wie auch die der Megaloblasten Leibetseder (1957) und Weicker haben mit Hilfe karyometrischer Analysen die kernhaltigen Normoblasten folgendermassen in funf Gruppen eingeteilt.

1) *Proerythroblasten* Kerndurchmesser 13 μ
2) *Makroblasten* Kerndurchmesser 10,3 μ
3) Basophile und polychromatische Erythroblasten Kerndurchmesser 8,2 μ
4) *Polychromatische Erythroblasten* Kerndurchmesser 6 5 μ
5) *Oxyphile Erythroblasten* Kerndurchmesser 5 2 μ

Die Zellen dieser letzten Gruppen stehen der reifen Form schon recht nahe ihr Kern ist stark pyknotisch unregelmässig gebaut und liegt meistens exzentrisch. Die nächste Reifestufe vor den regelrechten Erythrozyten sind die *Retikulozyten* In diesen Zellen ist kein eigentlicher Kern mehr wahrzunehmen und sie stehen den reifen Erythrozyten schon so nahe dass Undritz (1952 Hämatologische Tafeln Sandoz) sie als *Proerythrozyten* bezeichnet.

Das Hämoglobin

Das Hämoglobin ist ein aus zwei Komponenten nämlich der prosthetischen Gruppe und einem Eiweisskörper dem Globin zusammengesetztes Chromoproteid Das Hämoglobinkristall hat zum erstenmal Hünefeld 1840 dargestellt und später sind seine chemischen und physikalischen Eigenschaften eingehend beschrieben worden (Fischer et al. 19[illegible]7)

Der eigentliche Farbstoff des Hämoglobins die Hämgruppe macht nur 4 vom ganzen Hämoglobinmolekül aus Chemisch ist diese Farbkomponente eine Ferroverbindung von Protoporphyrin-IX worin das Eisen koordinativ 6-wertig ist. Die intrazellulär in den Erythrozyten des Knochenmarks stattfindende Porphyrinsynthese ist ein von Enzymen gesteuerter Prozess wo nach verschiedenen Phasen Protoporphyrin zustandekommt an dessen zwei periphere Kohlenstoffatome sich das Eisen binden kann

Die zweite Komponente des Hämoglobinmoleküls die [illegible] davon ausmacht, ist das zu den Albuminen gehörige Globin. Man nimmt an dass die Globinsynthese wie auch die des Porphyrins im Knochenmark vor sich geht, und zwar vermutlich in den Erythroblasten. Die Einzelheiten dieser Synthese sind jedoch bisher noch nicht völlig geklärt.

Nach den Untersuchungen von Thorell (19[illegible]7) sind der Nukleingehalt der Erythroblasten und die Globinbildung eng miteinander verknüpft. Ehe die Globinsynthese beginnt steigt der Nukleingehalt des Protoplasmas bis zum Sättigungsgrad an um dann abzusinken sobald die Globinsynthese einsetzt In dem Stadium wo sich Globin und Häm zum Hämoglobin verbinden ist die Nukleinsäure bereits nahezu verschwunden Die Bindung der Hämgruppe an das Globin geht offenbar nach dem Coulombschen Gesetz vor sich. Dieser Prozess lässt sich mit einer Oxydation des Moleküls vergleichen, wobei der Hämring sich gewissermassen an der Oberfläche des Eiweisskörpers anlagert.

Im reifen Erythrozyt wo diese »Oxydation» schon vollständig abgelaufen ist ist das intrazelluläre Hämoglobin gewissermassen inaktiv während wiederum in den nukleinsäurehaltigen Retikulozyten bei manchen pathologischen Zuständen sogar Aminosäurestoffwechsel stattfinden kann. Im Gegensatz zur Hämgruppe die sogar bei verschiedenen Tierarten identisch ist ist das Globin ausgesprochen artspezifisch und der Mensch z.B hat mehrere verschiedene Hämoglobintypen die sowohl elektrophoretisch wie auch immunologisch voneinander abweichen und deren Differenzen durch Variatio-

nen in der Eiweisstruktur des Globins bedingt sind (White & Beaven 1959 Allison 1958)

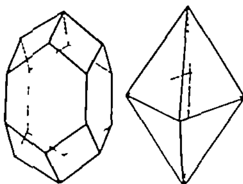


Fig 1 Fig 1b

Fig 1 Oxyhämoglobinkristalle a) Nabelschnurblutfarbstoff b) Erwachsenenblutfarbstoff

(Nach Beth)

Das fetale Hämoglobin

Die erste Angabe darüber dass man das fetale Hämoglobin sich vielleicht vom sog bleibenden Hämoglobin unterscheiden könnte stammt aus dem Jahre 1867 als Körber die Beobachtung machte dass das Plazentarblut gegen Alkalidenaturation resistenter ist als das Erwachsenenblut. Aber erst im Jahre 1910 gelang es Wakulenko mit Hilfe des gleichen Alkalidenaturationsverfahrens mit Sicherheit nachzuweisen dass der Nabelschnurblutfarbstoff vom Erwachsenenblutfarbstoff differiert.

Obwohl der Hämoglobinkristall bereits vor mehr als hundert Jahren beschrieben worden ist gelang die Kristallisation des fetalen Hämoglobins zum erstenmal erst im Jahre 1923 (Amantea)

Der Vergleich zwischen den obigen schematischen Darstellungen zeigt deutlich den Unterschied zwischen den Kristallen

Da die Differenzen zwischen den verschiedenen Hämoglobintypen in erster Linie vom Globin bedingt sind ist dieser Eiweisskörper begreiflicherweise sehr vielseitiger Forschung unterzogen worden v. d. Linden (1950) hat aus Analysen von Totalhydrolysaten signifikante Differenzen bezüglich des Vorkommens von Aminosäuren erhalten.

	Hb F	Hb A
Histidin	,3	,8
Isoleucin	1 6	0 5
Methionin	1 6	1,39
Prolin	4 1	4 8
Threonin	6 4	5 6
Tyrosin	2 8	3,1
Valin	9 1	10,2

Huisman (1955) und Mitarbeiter haben ebenfalls genaue Analysen von einigen wichtigsten Aminosäuren ausgeführt. Die Resultate sind aus der folgenden Zusammenstellung ersichtlich.

Aminosäure	Hb A	Hb F
Threonin	5 5	6,5
Serin	4 5	5 05
Glutaminsäure	7,25	7 65
Prolin	4 95	4,2
Alanin	9 95	9 7
Valin	10 9	9 5
Isoleucin	0 3	1 05
Tyrosin	3 45	3,2
Histidin	8,3	4

Die Resultate der beiden Untersuchungen stimmen überein abgesehen von kleineren quantitativen Unter

schieden Rhinesmith s (1958) Forscher gruppe hat nachgewiesen dass das menschliche Hämoglobin Molekül aus vier Polypeptidketten besteht, von denen je zwei und zwei identisch sind. Demgemäss wird HbA folgendermassen bezeichnet $\alpha_2\beta_2$. Hunt (1959) seinerseits hat festgestellt dass die Fetal hämoglobinkette deutlich von der ent sprechenden Kette des bleibenden Blut farbstoffs abweicht, und hat sie mit γ bezeichnet woraus sich für HbF die Bezeichnung $\alpha_2\gamma_2$ ergibt.

Nachdem die Forschungen und Beobachtungen von Turba (1934) und Tiselius (1937) es ermöglicht hatten, Proteine *elektrophoretisch* zu trennen sind auch verschiedene Hämoglobine von zahlreichen Autoren nach dieser Methode untersucht worden. Die Elektrophorese-Diagramme bei denen als Träger Filterpapier benutzt wurde das in bestimmter Pufferlösung behandelt war (Ionkonzentration des Puffers 0 03 —0 1 und pH etwa 6,5 und 8) liessen erkennen, dass das fetale Hämoglobin zur Anode langsamer und zur Kathode schneller wandert als das bleibende Hämoglobin (Beaven & al. 1959 Rich, 1952 Zinsser 1952)

Beaven und Gratzer haben fetales und bleibendes Hämoglobin voneinander getrennt wobei sie als Träger 1—1,5 %iges Agargel in 1/10 molarischer Pufferlösung mit einem pH von 6 0—6,5 benutzten. Zum gleichen Resultat kamen auch Owen & Got (1957) mit ihrer entsprechenden Stärke-Gel analyse

Auch *chromatographisch* lassen sich diese beiden Hämoglobine voneinander unterscheiden und zwar aufgrund der zuerst von Jope (1949) gemachten Beobachtung, dass im ultravioletten Gebiet die Kurve des HbF deutlich von der Kurve des HbA abweicht. Der Schwerpunkt der Abweichung liegt bei 289 μ

Da der fetale und der bleibende Blut farbstoff zwei verschiedene Eiweisskörper sind ist es klar dass man auch ihre *serologischen* Verschiedenheiten untersucht hat. Als Erster hat sich Darrow (1940) mit diesen Fragen befasst er immunisierte Kaninchen mit durch Aluminiumhydroxyd gereinigtem Hämolysat von Fetal- und Erwachsenen blut. Später haben insbesondere die immunologischen Untersuchungen von Chernoff (1953) weitere Aufschlüsse zu dieser Frage geliefert. Es gelang ihm mit gereinigtem HbF die Bildung von Anti F Serum herbeizuführen. Dies stützt die Auffassung von Darrow dass der fetale Blutfarbstoff eine spezifische Antikörperbildung zu standebringen könne.

Die ganze Unterscheidung des fetalen Hämoglobins vom bleibenden geht ursprünglich auf die Beobachtung zurück dass das erstere resistenter gegen Alkalidenaturation ist als das letztere Die Untersuchungen von Bischoff & Schulte (1926) sowie Haurowitz (1930) haben diese Frage näher aufgeklärt und den Boden für die umfassende Forschungsarbeit geschaffen die diese Eigenschaft sowohl für qualitative wie quantitative Bestimmungen ausgenutzt hat. Von diesen auf der Alkalidenaturation beruhenden Methoden gibt es verschiedene Modifikationen von denen aber die von Brinkman & Jonxis (1935 1937) Singer Chernoff & Sin-

ger (1951) am üblichsten sind. Alle sind exakte und zuverlässige Verfah ren, aber alle haben den Nachteil dass die quantitative Genauigkeit, wenn es sich um kleine Quanten HbF handelt, nicht die bestmögliche ist. Die von Beaven Ellis & White (1960) entwick elte Kombinationsmethode die spek trographische Alkalidenaturation er möglicht die Feststellung auch klei ner Quanten.

Gemeinsam für alle diese Verfahren ist jedoch dass das Hamoglobin in Lösung m.a.W als Hämolysat auftritt Im Rahmen einzelner Erythrozyten ist die Untersuchung des Fetalhämoglobins mit Hilfe dieser Methoden na türlich nicht möglich. Kleihauer Braun und Betke (1957) haben nach der Möglichkeit gesucht, die Fetalhamoglobin enthaltenden Erythrozyten von denjenigen zu trennen, die bleibendes Hämoglobin enthalten Sie untersuchten die Löslichkeit der Erythrozyten unter der Einwirkung von Enzymen und mach ten die Beobachtung, dass Pepsin im fixierten Blutpräparat das Erwachsenenhämoglobin erheblich schneller löst als im Nabelschnurblutpräparat das hauptsächlich Fetalhamoglobin enthält. Betke (1958) setzte seine Versuche fort und fand dass das Resultat das gleiche war wenn anstelle von Enzymen Zitronensaurephosphatpufferlösung mit pH 3 4 benutzt wurde Mit diesem Verfah ren konnten Betke und Kleihauer (1958) ein Färbungspräparat herstellen, wo in den Erythrozyten das bleibende Ha moglobin sich gelöst hatte, und die Zellen im Mikroskop als leere Hüllen zu sehen waren während wiederum das Fetalhämoglobin ungelöst zuruck geblieben war und die Zellen sich schön rot färbten.

Wir haben also die Möglichkeit, das fetale Hämoglobin und sein Vorkom men im ganzen mit Hilfe der Alkali denaturation zu untersuchen oder wir können seine elektrophoretischen spek trophotometrischen und serologisch immunologischen Eigenschaften aus nutzen Wenn wir sein Vorkommen im Rahmen einzelner Erythrozyten untersuchen wollen können wir mit dem von Kleihauer Betke entwickelten Elu tions-Färbeverfahren im fixierten Prä parat die Erythrozyten herausbekom men die Fetalhämoglobin enthalten sowie auch die welche bleibendes Hä moglobin einschliessen.

ZWECK DER UNTERSUCHUNG, MATERIAL UND METHODE

Die vorliegende Untersuchung hatte den Zweck, anhand von gefärbten Präparaten zu verfolgen, wie sich fetales und bleibendes Hämoglobin in den roten Blutzellen während der fetalen Entwicklung verteilen. Hierfür wurden Blutproben von Feten in verschiedenen Stadien der intrauterinen Entwicklung genommen und nach der von Kleihauer-Betke entwickelten Elutions-Färbemethode untersucht. Ferner sollte versucht werden, wenn möglich, den Boden zu schaffen für weitere Untersuchungen, die auf die Lösung der obstetrischen Probleme hinzielen, bei denen das pathologische Auftreten von Fetalhämoglobin vermutlich eine Rolle spielt. Hierbei kommt zunächst der Nachweis von aus den Feten herstammenden fetalhämoglobinhaltigen Erythrozyten in Frage, wenn solche plötzlich, z. B. in Fällen von Plazentaläsion, in den mütterlichen Kreislauf gelangen. Auch bei Blutungen gegen Ende der Schwangerschaft kann die Kenntnis der morphologischen und vor allem der färberischen Eigenschaften der fetalen Erythrozyten zur richtigen Diagnose verhelfen, wenn es zu entscheiden gilt, ob die Blutung mütterlicher oder fetaler Herkunft ist. Ferner wurden bei der Untersuchung die Möglichkeiten in Betracht gezogen, die uns die mit dieser Elutions-Färbemethode erzielten Resultate für die Bestimmung des Entwicklungsalters der Frucht bieten.

Material und Methode

Das Material wurde in den gynäkologischen und obstetrischen Abteilungen der I. Universitäts-Frauenklinik in Helsinki gesammelt. Die Proben aus den ersten Fetalmonaten wurden bei Fällen von Sectio caesarea minor erhalten. Bei drei Monate alten und jüngeren Feten wurde die Blutprobe aus Herz und Leber genommen. Vom vierten Fetalmonat an wurden die Proben durch direktes Einträufeln des Blutes aus der Nabelschnur in das Reagenzglas gewonnen. Alle Proben von fünfmonatigen und älteren Feten wurden von lebend geborenen Feten im Entbindungssaal genommen.

Das Material umfasst 343 Proben, die nach den Fetalmonaten gruppiert sind. Das Alter der Frucht wurde nach

der Menstruation berechnet, indem zum Tag des Beginns der letzten Menstruation 14 Tage hinzugezählt wurden und von dem so erhaltenen wahrscheinlichen Tag der Konzeption wurde das Alter der Frucht auf eine Woche genau bestimmt. Die Gruppierung in Monate wurden so vorgenommen, dass zur gleichen Monatsgruppe die ± 1 Woche davon abweichenden Fälle gerechnet wurde. So gehören z.B. zur Gruppe der 4 Monate alten die 15, 16 und 17 Wochen alten Feten. Eine Ausnahme von der allgemeinen Gruppierung bildet die erste Gruppe der 3 Monate alten Früchte. Hierzu gehören ausser den 11, 12 und 13 Wochen alten nur zwei 9 Wochen alte und ein 10 Wochen alter Fetus. Die Fälle verteilen sich folgendermassen:

Alter der Feten	*Anzahl der Fälle*
3 Monate	28
4	39
5	31
6	28
7	24
8	36
9	3
10	15

Die Serie beginnt mit einem 9 Wochen alten 2,3 cm langen Fetus und schliesst mit einem 54 cm langen 5050 g schweren Neugeborenen ab. Jedem gefärbten Präparat entspricht ein Kontrollpräparat, das aus der Blutprobe einer nicht graviden Frau hergestellt und zugleich mit der Fetalprobe gefärbt wurde. Auf diese Weise konnten sofort alle Fehler erfasst werden, die eventuell im Färbeverfahren auftraten und zunächst durch pH-Schwankungen der Pufferlösung verursacht waren. Ausserdem war es dadurch möglich, laufend die in den Fetalproben vorkommenden bleibendes Hämoglobin enthaltenden Zellen und deren Färbbarkeit mit den entsprechenden Erythrozyten des erwachsenen Menschen zu vergleichen. Die an der Luft getrockneten Ausstrichpräparate wurden alle innerhalb von sechs Stunden nach der Probeentnahme behandelt. Alle pathologischen Fälle wurden zu eliminieren versucht, weil möglichst der Normalsituation entsprechende Resultate angestrebt wurden. Jedes Ausstrichpräparat wurde mikroskopisch untersucht und auf jedem Gläschen 2000 Erythrozyten abgezählt. Beim Zählen wurde einheitlich folgendes Verfahren befolgt. Der Objektträger wurde in zwei gleiche Hälften eingeteilt und beiderseits der Mittellinie wurden zum Rande hin 1000 Zellen abgezählt. An den beiden langen Rändern des Objektträgers wurden den Streifen von ca. 2 mm ausser acht gelassen, um die Wirkung der Randverdichtung zu eliminieren.

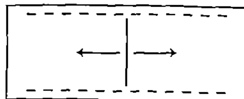


Fig. 2. Schematische Darstellung vom Zellenzählgebiet auf dem Objektträger

Die Zählung wurde ferner noch so kontrolliert, dass beiderseits der Mittellinie vier nebeneinander gelegene Felder genommen und dort die Zellen abgezählt wurden. Die Resultate stimmten

prozentual überein. Die Zellenzählung wurde insofern blind vorgenommen, als die Gläschen nur die laufende Nummer trugen. Es war dem Zähler also nicht bekannt, wie alt der Fetus war, von dem die Probe stammte.

Die Blutproben wurden in ein Reagenzgläschen genommen, auf dessen Boden sich schon vorher ein Tropfen Heparinlösung befand. Die Probe wurde mit physiologischer Kochsalzlösung soweit verdünnt, dass auf 1 Teil Blut 3 Teile Kochsalzlösung kamen. Mit der Mikropipette wurde nun 1 ml von dieser Verdünnung aufgesaugt und auf den Objektträger getropft. Der Tropfen wurde in üblicher Weise möglichst dünn über das ganze Gläschen verteilt. Nachdem der Ausstrich mindestens 2—3 Min. lang getrocknet war, wurde die Probe zur Fixierung 5 Min. lang in 80 %igem Alkohol gehalten. Danach wurde der Objektträger sorgfältig unter langsam fliessendem Wasser 5 Min. lang gespült, sodann getrocknet und für 5 Min. senkrecht in eine Glasschale mit Pufferlösung gestellt. Zur Förderung des Lösevorgangs wurde der Objektträger nach Verlauf von 1 und 3 Min. in der Schale hochgehoben. Nach der Pufferung wurde das Gläschen nochmals 2 Min. lang in fliessendem Wasser gespült. Zur Färbung wurde die Probe 3 Min. lang in *Hämatoxylinlösung* gelegt, in fliessendem Wasser gewaschen und danach noch 3 Min. lang in *Erythrosinlösung* (Merck 0.1 %) gehalten. Schliesslich wurde das Präparat noch mit Wasser gespült und getrocknet, wonach es fertig war für die mikroskopische Untersuchung (Beilage Bild 1 u. Farbfoto).

Die Pufferlösung wurde folgendermassen hergestellt. Zwei Lösungen: A = 0,2 n $NaHPO$ und B = 0,1 n Zitronensäure. Es werden 26,6 ml der Lösung A mit 73,4 ml der Lösung B vermischt. pH dieser Lösung müsste dem geforderten Wert von 3,3 entsprechen, aber dies muss stets mit dem pH-Messer kontrolliert werden. Die Temperatur der Pufferlösung muss während des Lösens ständig bei 37° C bleiben, weshalb die Schale mit dem Puffer im Voraus in ein Wasserbad von konstanter Temperatur gestellt wurde.

Bei der Anwendung dieses Elutionsfärbeverfahrens müssen die Vorschriften streng befolgt werden. Der grundlegende Faktor der ganzen Methode ist natürlich der lösende Puffer. Sein pH muss 3,3 sein. Bei höheren pH-Werten kommt es nur zu teilweiser Auflösung, und dann farben sich auch die bleibendes Hämoglobin enthaltenden Zellen an, während wiederum bei niedrigeren pH-Werten sich auch das fetale Hämoglobin auflöst. Kleihauer (1962) hat Versuche mit wechselnden pH-Werten gemacht, und seine Resultate sind aus den beigefügten Abbildungen ersichtlich (Beilage Bild 2).

Bei den von mir ausgeführten Kontrollen ergaben sich genau die gleichen Ergebnisse. Kleihauer hat ferner beobachtet, dass auch die Frische der Probe ihre Bedeutung hat. Wenn die Probe ein über 12 Stunden altes, an der Luft getrocknetes Ausstrichpräparat ist, sollte die Alkoholfixation auf 1—2 Minuten herabgesetzt werden. Der beste pH-Wert ist dann 3,2.

Eine wichtige Fehlerquelle ist die Temperatur des Puffers. Unter 20 C ist die Lösung nicht vollständig und auch die Zellen mit bleibendem Hämoglobin färben sich an obschon sie heller bleiben als die Fetalzellen.

Beim Ausprobieren der Methode wurden immer wieder unbefriedigende Serien *erhalten obwohl die wichtigsten* Faktoren, pH und Temperatur von 37 C peinlich eingehalten wurden. Aus diesem Grunde wurden alle möglichen Fehlerquellen Punkt für Punkt durchgegangen. Zuerst wurde kontrolliert welchen Einfluss die zur Verdünnung benutzte Kochsalzlösung auf das Gelingen der Präparate hatte. 0,9 %ige NaCl-Lösung wurde über Nacht in einem offenen Gefäss stehengelassen, und am nächsten Morgen wurde diese Lösung zum Verdünnen der Blutproben benutzt. Dabei wurden in zwei Proben von Männern und vier Proben von nicht graviden Frauen viele Erythrozyten gefunden die sich wie die Fetalhämoglobinzellen farbten Aufgrund dieser Beobachtung wurde eine Serie hergestellt, in der Kochsalzlösungen von verschiedener Konzentration nämlich 0,5 0 9 1 ? und 4 % benutzt wurden. Bei der 1 %igen Lösung trat schon die Erscheinung zutage dass das bleibende Hämoglobin nicht gänzlich aufgelöst wurde. Wenn 0,5 %ige Lösung verwendet wurde waren deutlich Zeichen von Hämolysierung der Zellen zu sehen. *Mit 2 %iger und* 4 %iger Kochsalzlösung hämolysierten sich die Zellen im direkten Verhältnis zur Konzentration (Beilage, Bild 3)

Um zu ermitteln welche Wirkung eine eventuelle Oxydation der Proben auf die Resultate hätte, wurde fünf Minuten lang reiner Sauerstoff in die Blutproben geleitet was aber keinen Einfluss auf die Löslichkeit und Färbbarkeit hatte. Es spielt somit keine Rolle ob bei der Elutionsfärbung die Probe arterielles oder venöses Blut ist.

*Die für das Gelingen des Elutionsfär*beverfahrens ausschlaggebenden Punkte lassen sich kurz folgendermassen zusammenfassen.

1) pH der Pufferlösung muss 3,3 sein Wenn das Präparat über 6 Stunden alt ist soll pH 3 2 sein. Die Pufferlösung muss jeden Tag neu hergestellt werden.
2) Die beste Temperatur für die Pufferlösung ist 37 C.
3) Die zur Verdünnung benutzte Kochsalzlosung muss genau physiologisch sein und für jede Färbung soll die neue physiologische Kochsalzlösung kontrolliert werden.

Da die Methode auf der verschiedenartigen Löslichkeit der Hämoglobine beruht, ist bei der Beurteilung dieses intrazellulären Prozesses die Frage berechtigt welche Rolle die Zellpermeabilität in diesem Vorgang spielt. Die Untersuchungen von *Schuboths* (1957) zeigen jedoch dass diese Erscheinung nichts mit der Zellpermeabilität zu tun hat. Er benutzte Hämolysat, aus *dem das Stroma* abgefiltert worden war und bekam mit Erwachsenen- und Nabelschnurhämoglobin völlig voneinander abweichende Extinktionskurven

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Verteilung auf Zellgruppen

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Obschon diese Einteilung in fünf Erythrozytengruppen in erster Linie aufgrund der Färbbarkeit vorgenommen worden ist wurden zugleich auch die morphologischen Unterschiede in Betracht gezogen.

Beim normalen Erythrozyt schwankt die Länge des Durchmessers nach verschiedenen Autoren zwischen 7 15 μ und 8 μ Messungen an den mütterlichen Erythrozyten in der Schwangerschaft haben ergeben dass der Mittelwert des Durchmessers in den zwei ersten Dritteln der Gravidität 7 5 μ beträgt und Ende des letzten Drittels oder bei der Entbindung entsprechend 7 7 μ (Tjan und Oes 1 [illegible]) Hansler und Riegel ([illegible]) haben die fetalen Erythrozyten gemessen und sind zu folgenden Werten gekommen

	10 Fetalwoche	22 Fetalwoche	Ausgetragen
Durchmesser	11 μ	8 0 μ	7 9 μ

Man sieht dass der Durchmesser des fetalen roten Blutkörperchens auch noch beim ausgetragenen Kind grösser ist als der Durchmesser der mütterlichen roten Blutzelle ganz zu schweigen von den früheren Stadien der Fetalentwicklung

Die gleiche Erscheinung wie in den Untersuchungen von Hansler und Riegel lässt sich auch in meinem Material im Laufe der fetalen Entwicklung feststellen. Die Grösse der fetalen Erythrozyten nimmt mit zunehmendem Alter der Frucht ab aber auch noch beim ausgetragenen Kind sind die Erythrozyten grösser als die sog normalen Erwachsenenerythrozyten

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kernhaltig Nur etwa 10 von ihnen sind noch kernlos und diese kernlosen Zellen wurden aufgrund ihrer Grösse zu der ersten Gruppe gerechnet. Sie entsprechen offenbar den Zellen der megaloblastischen Periode die Mundorff als *Megalozyten* bezeichnet Die Farbintensität des sich rot anfärbenden Hämoglobins ist nicht gleichmässig man bekommt vielmehr den Eindruck als ob das Hamoglobin stellenweise ausgeflockt ware (Beilage Bild 4)

Die Zellen der *Gruppe II* sind kleiner als die obigen, sie haben aber doch noch einen mittleren Durchmesser von 1—10 μ Die Zellstruktur ist homogen die Zellen haben keinen Kern. Im Innern der Zellhulle sieht man ziemlich stark gefärbte wurmförmige Gebilde und zwischen diesen schmale ungefarbte Gebiete Das gefärbte Gebiet macht von der ganzen Fläche der Zelle mehr als 50 % aus. (Beilage Bild 5)

Die Zellen der *Gruppe III* stehen morphologisch den regelrechten Erythrozyten schon nahe Sie sind kernlos und haben einen mittleren Durchmesser von 8—9 μ Die Struktur ist klar ausgeprägt und homogen Die ganze Zelle farbt sich durchweg gleichmässig rot.

Die Zellen der *Gruppe IV* kommen hinsichtlich Grösse und Struktur etwa denjenigen der dritten Gruppe gleich. Der Unterschied liegt nur in der Farbbarkeit. Die Zellen sind rosa und zwischen den gefarbten Gebieten liegen vollig farblose Stellen diese farblosen Gebiete nehmen an der Zelloberfläche den grössten Teil ein (Beilage Bild 6)

Die Zellen der *Gruppe V* sind dann schon mit den reifen Erythrozyten identisch ihr Durchmesser ist durchschnittlich 8 μ und im gefarbten Präparat sind sie als fast leere farblose Hullen zu sehen.

Deutung der Zellgruppen und ihre Frequenzschwankungen im Lauf der Fetalentwicklung

Gruppe I (Primitive Erythrozyten HbFp) Diese Zellen der Gruppe I differieren morphologisch unverkennbar von den anderen Erythrozyten und sie erinnern weitgehend an die Zellen der mesoblastischen Periode Im hämatopoetischen System sollen die mesoblastischen Zellen ubiquitär im Dottersack gebildet werden und sie sind somit nicht an die blutbildenden Organe gebunden. Da man diese gewissermassen unspezifischen mesoblastischen Zellen in der Gesamthämatopoese als eine Art primitiver Erythrozyten betrachten kann wäre es denkbar dass auch das in ihnen enthaltene Hämoglobin irgendwie unreif also primitiv wäre

Künzer (1957) hat nachgewiesen, dass die Alkalidenaturationskurven von den Blutproben neunwöchiger und zwanzigwöchiger Feten deutlich voneinander abweichen. Auch mit Agarelektrophorese wurden ähnliche Resultate erzielt (Butler u. Mitarb 1960) und desgleichen mit Papierelektrophorese (Halbrecht und Klibanski 1956 sowie Zilliacus 1960) Aus Diagramm 1 ist ersichtlich dass der Frequenzgipfel dieser Zellen der Gruppe I die hier mit HbFp bezeichnet sind, in die ersten Fetalwochen fällt, wonach die Frequenz bis zum fünften Fetalmonat ziemlich steil ab-

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	10. Fetalwoche	[illegible] Fetalwoche	Ausgetragen
Durchmesser	11 μ	8 6 μ	9 μ

Man sieht, dass der Durchmesser des fetalen roten Blutkörperchens auch noch beim ausgetragenen Kind grösser ist als der Durchmesser der mütterlichen roten Blutzelle, ganz zu schweigen von den früheren Stadien der Fetalentwicklung.

Die gleiche Erscheinung wie in den Untersuchungen von Hanssler und Riegel lässt sich auch in meinem Material im Laufe der fetalen Entwicklung feststellen. Die Grösse der fetalen Erythrozyten nimmt mit zunehmendem Alter der Frucht ab, aber auch noch beim ausgetragenen Kind sind die Erythrozyten grösser als die sog. normalen Erwachsenenerythrozyten.

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bezeichnet habe) in den vierten Fetalmonat fällt wonach die Frequenz ziemlich gleichmässig bis zum Ende der Fetalentwicklung abnimmt. Im dritten Fetalmonat ist die Frequenz (18 %) fast ebenso niedrig wie beim ausgetragenen Kind (12 %). Diese Beobachtung wirkt zunächst überraschend insbesondere da die Frequenz im vierten Fetalmonat nicht weniger als 44 % beträgt. Es lässt sich dies m.E. aber dadurch erklären dass im Laufe des dritten Fetalmonats in der Hämatopoese fast ausschliesslich das mesoblastische System dominiert wenn die Bildung von bleibendem Hämoglobin sehr geringfügig ist. Dementsprechend ist auch der Anteil der Mischzellen die bleibendes Hämoglobin enthalten klein. Während des vierten Fetalmonats hat schon in der Hauptsache der Übergang zum hepatolienalen System stattgefunden, wo bleibendes Hämoglobin wesentlich mehr gebildet wird und hieraus folgt auch dass Mischzellen jetzt zahlreicher auftreten als im vorherigen Fetalmonat.

Man kann sich auch fragen, warum der Anteil dieser Mischzellen im vierten Monat so hoch ist nämlich 44 %, während er schon im Lauf des fünften und sechsten Fetalmonats auf etwa 30 % absinkt. Es wäre denkbar dass in diesem Stadium die roten Blutzellen hauptsächlich fetales Hämoglobin bilden, während die geringere Synthese des bleibenden Blutfarbstoffs fast ausschliesslich an diese Mischzellen gebunden wäre. Mit dem Fortgang der Entwicklung der Frucht geht auch die Hämoglobinbildung allmählich zur bleibenden Form über und dann ist es ganz natürlich dass der Anteil dieser teilweise unreifen vorwiegend Fetal hämoglobin enthaltenden Mischzellen entsprechend zurückgeht.

Gruppe III (Ausschliesslich Fetalhämoglobin enthaltende Erythrozyten HbF). Die gleichmässig gefärbten Erythrozyten der Gruppe III enthalten ausschliesslich fetalen Blutfarbstoff. Aus Diagramm 3 ist die Frequenz dieser Zellen in den verschiedenen Fetalmonaten ersichtlich. Die im übrigen ziemlich ebenmässige Kurve wird im vierten Monat von der Prozentzahl 45 unterbrochen die deutlich unter dem allgemeinen Niveau liegt. Diese Abnahme stimmt jedoch gut mit der Frequenz der Mischzellen der vorigen Gruppe und damit überein dass offenbar eben in diesem Stadium in der Hämoglobinsynthese die Grenze überschritten wird, und reine Zellen treten dann weniger auf als diese Grenz oder Mischzellen.

Vom siebten Fetalmonat an weist die Kurve eine fortlaufend sinkende Tendenz auf und hierin spiegelt sich die bekannte Tatsache wider dass der Anteil des fetalen Hämoglobins an der Gesamtblutmenge eben im Lauf der letzten Fetalwochen am meisten abnimmt.

Die von verschiedenen Forschern mit der Alkalidenaturation durchgeführten Messungen des Vorkommens von Fetalhämoglobin im Lauf der Fetalentwicklung haben im grossen und ganzen übereinstimmende Resultate ergeben und die folgende, von Betke formulierte schematische Darstellung zeigt die Entwicklung im Licht der Alkalidenaturationsresultate.



Fig 3 Verhalten von HbF und HbA im Ablauf der Fetalentwicklung und den ersten Monaten nach der Geburt (Nach Betke)

Man sieht dass der Anteil des fetalen Blutfarbstoffs bis zum achten Fetalmonat ziemlich konstant ist nämlich etwa 90 / wonach er im Lauf der zwei letzten Monate auf ungefähr 70 / absinkt. Vergleicht man die aus Diagramm 3 ersichtlichen Prozentzahlen meines Materials mit dieser schematischen Darstellung, so ergibt sich *dass sie durchweg niedriger sind als die* Werte der Alkalidenaturation. Hier ist jedoch zu bedenken dass die Alkalidenaturation ein Gesamtbild vermittelt während wieder in den gefärbten Präparaten das Fetalhämoglobin sich ausser auf die Zellen der Gruppe III noch auf die Mischzellen verteilt.

Gruppe IV (Mischzellen in denen das bleibende Hämoglobin vorwiegt HbA + HbF) Die Zellen der Gruppe IV stellen den vielleicht am schwersten zu deutenden Teil des ganzen Materials dar Sie sind deutlich heller als die Zellen der Gruppe III und differieren auch wesentlich von den Mischzellen der Gruppe II

Betrachtet man den Sachverhalt auf dem Boden der von Kleihauer und Betke dargelegten Mischzellentheorie kann man diese Erythrozyten den Zellen der Gruppe II an die Seite stellen jedoch mit dem Unterschied dass in den erstgenannten das bleibende Hämoglobin die Mehrheit ausmacht Wenn wir nun wieder zur Gesamthämatopoese zurückkehren so stellen wir fest dass die myeloische Periode im fünften Fetalmonat einsetzt dass ihre Beteiligung aber erst nach dem siebten Monat *ausschlaggebend ansteigt. Obschon* bleibendes Hämoglobin bis zu einem gewissen Grad in allen Phasen der Hämatopoese gebildet wird ist es doch ganz evident dass die Produktion hauptsächlich in den Erythrozyten des Knochenmarks stattfindet.

Aus Diagramm 4 lässt sich entnehmen dass die Kurve die den prozentualen Anteil dieser HbA + HbF Erythrozyten veranschaulicht in den ersten Fetalmonaten ziemlich niedrig bei 4—6 % verläuft und dass der steile Anstieg erst im achten Monat einsetzt. Diese Kurve ist der den Anteil der myeloischen Hämatopoese anzeigenden Kurve analog und dieser Umstand verlockt natürlich zur Akzeptation des Gedankens dass diese rosa gefärbten Erythrozyten eben die Mischzellen wären die vorwiegend bleibendes Hämoglobin enthalten Kompli

z ert wird die Sache jedoch durch die Beobachtung dass es auch fetalhamoglobinhaltige Retikulozyten geben soll (Kleihauer 1960) Beilage Bild ,

Wie aus dem Bild hervorgeht ver halten die bleibendes Hamoglobin ent haltenden Retikulozyten sich zur Auf lösung genau so wie die reifen Erythrozyten so dass also in dem Elutionsfärbeverfahren diese Retikulozyten kein Problem darstellen. Dahingegen ware es schwierig möglicherweise Fetalhämoglobin enthaltende Retikulozyten von solchen roten Blutzellen zu unter scheiden die sowohl bleibendes wie auch fetales Hämoglobin fuhren.

Zu bemerken ist jedoch dass sich in diesen rosa angefärbten Zellen abgesehen von wenigen Ausnahmen keine fur die Retikulozyten charakteristischen Kernreststrukturen nachweisen liessen. Dies spricht gegen die Annahme dass es sich um fetale Retikulozyten handle aber andererseits kann man nicht in Abrede stellen dass mit spezieller Retikulozytenfarbung manche Zellen zum Vorschein gekommen sind deren Struk tur in gewissem Masse an die Retikulozyten erinnerte. Weitaus die meisten von diesen Zellen besitzen diese Nei gung jedoch nicht und man darf sie daher wohl als eine Mischzellgruppe für sich behandeln

Gruppe V (Ausschliesslich bleibendes Hamoglobin enthaltende Zellen HbA) Walker und Turnbull (1955) ha ben verschwindend kleine Mengen bleibenden Hämoglobins bei einem 13 Wochen alten Fetus gefunden. Vom funften bis zum achten Monat bleiben ihre mit Alkalidenaturation erhaltenen Werte bei etwa 10 % und dann steigen sie bis zum Abschluss der Fetalentwick lung gleichmässig bis auf 30 % an. Ponder und Levine (19 9) teilen für den ausgetragenen Fetus Werte von 20—30 und Haurovits (1935) sogar 20—40 mit Alle diese Zahlen geben die Gesamtmenge des bleibenden Hämoglobins an. Alle haben gemeinsam dass der Anteil des bleibenden Blutfarbstoffs erst vom achten Fetal monat an zunimmt.

In meinem Material sind zwei neun wöchige Feten enthalten, in denen wir Erythrozyten mit bleibendem Hämoglobin gefunden haben. Wie aus Dia gramm 5 ersichtlich ist wurde bleibendes Hämoglobin durchweg im ganzen Material angetroffen. Vom dritten bis zum sechsten Fetalmonat ist die Menge des bleibenden Blutfarbstoffs minimal und in den Diagrammen ist dieses Quantum mit <1 angegeben. Im siebten und achten Monat ist die Menge 1 Dann setzt ein steiler Anstieg ein der beim ausgetragenen Kind mit dem Medianwert 11 , abschliesst Der niedrigste Wert beim ausgetragenen Fetus war 7 %, und auch dieser kam nur einmal vor

Als Besonderheit möge erwähnt sein dass sich in der Gruppe der ausgetragenen Früchte acht befanden deren Geburtsgewicht über 4 500 g betrug Berechnet man fur diese Gruppe ge sondert den Medianwert fur das bleibende Hamoglobin, so ergibt sich 19 % — Vergleicht man nun diese Ergebnisse mit den Resultaten der Alkalidenaturation, so sieht man dass die letzteren durchweg deutlich höher liegen. Wah

rend die Werte bei der Alkalidenaturation vom fünften bis zum achten Monat etwa 10 % betragen steigen sie in meinem Material nicht über 1 %. Die Differenz ist gross sie wird aber weitgehend ausgeglichen wenn wir das in den Mischzellen enthaltene bleibende Hämoglobin der entsprechenden Zeit berücksichtigen. Beim ausgetragenen Fetus ist das Alkalidenaturationsniveau etwa 30 %, während der entsprechende Wert in meinem Material nach der Färbemethode berechnet nur 11 % beträgt. Die Existenz der Mischzellen erklärt diesen prozentuellen Unterschied.

Verteilung der Zellen auf die Gruppen im Lauf der verschiedenen Fetalmonate

Dreimonatige Feten.

Die Kurve der Medianwerte in Diagramm 6 zeigt wie die Erythrozyten sich in diesem Stadium der Fetalentwicklung auf die Gruppen verteilen. Die Streuung in den oberen und unteren Quartilen ist gering. In der Streuungstabelle (Tabelle 1) sind diejenigen Streuwerte angegeben die ausserhalb der Quartile liegen.

Tabelle 1. Die Zellen der ausserhalb der Quartile bleibenden Erythrozyten bei den dreimonatigen Feten

Gruppe I		Gruppe II		Gruppe III		Gruppe IV		Gruppe V	
	Zahl		Zahl		Zahl		Zahl		Zahl
9	1			4	2	6	1	—	—
19	4	16		64		—	—	—	—
14		13	3	60		—	—	—	
13	1	14	1	59	1	—	—	—	—

Da es sehr interessant ist, in welchem Verhältnis die verschiedenen Zellgruppen in den Proben mit Extremwerten zueinander stehen, habe ich die entsprechenden Werte der anderen Zellgruppen in diesen Extremwertfällen der Feten verschiedenen Alters in Serien für sich zusammengestellt.

Zellen der Gruppe I. In den Extremwertfällen verteilten die Zellgruppen sich folgendermassen

V	I	II	III	IV	V
1	20	1	9	6 %	< 1 %
2	19	1	60	4	< 1
3	19	18	60	3	< 1
4	19	14	62	5 %	< 1 %
5	19	15	62 %	4	< 1
6	14	18	63	6	< 1 %
	14 %	18	6		< 1
8	13	[illegible]	64	3	< 1

In dieser Gruppe ist keine dahingehende Korrelation wahrzunehmen, dass die Abnahme der primitiven Zellen unter das Medianniveau das Bild unbedingt nach rechts in reifer Richtung verschiebe. Bezüglich der Zellen der Gruppe V lässt sich nichts sagen und auch die nächste Gruppe IV ist in dieser Hinsicht ganz inhomogen. Nur in Gruppe III kann man eine gewisse Steigungstendenz bei der Minderung der Primitivzellen beobachten.

Zellen der Gruppe II. Den Extremwerten der Zellen dieser Gruppe gemäss verteilen die Zellgruppen sich folgendermassen.

Die Veränderungen in den Zellen der Gruppe II scheinen zunächst mit den Zellen der Gruppe IV insofern zu

N	I	II	III	IV	V
1	18 %	0	62	3	<1
2	18	0	62	3	<1
3	13	20	64	3	<1
4	16	20	61	3	<1
5	17	16	8	3	<1
6	16	16	64	4	<1
7	20 %	15	59	6	<1
8	19 %	15	62	4	<1
9	16	18	63	4	<1
10	19	14	6	5	<1

korrelieren, als den Maximalwerten der ersteren Gruppe die Minimalwerte der letzteren entsprechen und die den Minimalwerten der Gruppe II entsprechenden Werte der Gruppe IV bevorzugt unterhalb der Mediangrenze liegen. Die Differenzen sind jedoch so uneinheitlich dass sich keine statistische Signifikanz ergibt.

Zellen der Gruppe III In dieser Gruppe sind die Zellverteilungen der Extremwerte folgende

N	I	II	III	IV	V
1	14 %	18	65	4	<1
2	1	18	65 %	3	<1
3	16	16	64 %	4	<1
4	13 %	20	61	3	<1
5	19	1	60	4	<1
6	19	18	60	3	<1
	20	15	59	6	<1

Die Frequenz der Minimalwerte der Zellen der Gruppe III und die Frequenz der entsprechenden Primitivzellen weisen im Rahmen dieser Gruppierung gleiche Richtung auf In den Extremwertfällen der Primitivzellen entspricht in drei Proben den hohen Primitivzellwerten ein unterhalb vom unteren Quartil liegender Wert aber in zwei Fällen liegt die Gruppe III in der Medianebene Da die Richtung jedoch in der Hauptsache sowohl bei den Primitivzellen wie auch bei den Zellen der Gruppe III die gleiche ist kann man aufgrund dessen zumindest sagen dass beim dreimonatigen Fetus die Verminderung der Primitivzellen sich zunächst als Veränderungen in der Gruppe III widerspiegeln. Man kann dies vielleicht als ein Zeichen von Reifung auffassen.

Zellen der Gruppe IV In der Gruppe IV ist keine wesentlichere Streuung wahrzunehmen. Nur ein einziger Wert nämlich 6 %, weicht vom Quartilgebiet ab Er repräsentiert den Fall, in dem die Primitivzellen 20 % ausmachten in dieser Probe differieren alle Werte vom übrigen Niveau.

Bei den *Zellen der Gruppe V* treten überhaupt keine Schwankungen auf ihre Frequenz ist in allen Proben minimal.

Viermonatige Feten.

Bei den viermonatigen Feten weicht die Mediankurve welche die Verteilung der Zellgruppen veranschaulicht von allen anderen Monatskurven ab Ihr flacher Gipfel (Diagramm 7) kommt nicht einmal mit dem höchsten Extremwert an die Minimalwerte der benachbarten Kurven heran. Darüber wie dieser flache Gipfel zustandegekommen ist lassen sich verschiedenerlei Vermutungen vorbringen.

Im Vergleich zum vorangegangenen Monat hat sich die Zahl der Zellen der Gruppe II mehr als verdoppelt während wiederum die Zellen der Gruppe

III sich um etwa 30 vermindert haben um im fünften Monat erneut auf das frühere Niveau anzusteigen

Man kann bei seinen Überlegungen davon ausgehen dass gerade zu diesem Zeitpunkt in der Gesamthämatopoese eine umwälzende Wandlung eintritt denn nun kommt ja die hauptsächlich an die Organe gebundene zuvörderst in der Leber stattfindende Blutbildung in Gang Die Produktion der Primitivzellen ist ganz minimal Auch in den gefärbten Präparaten ist ihr Anteil auf die Hälfte im Vergleich zum vorherigen Monat gefallen und trotzdem darf man hier nicht vergessen dass von den Zellen die im gefärbten Präparat der viermonatigen Feten zu sehen sind ein Teil zu den im dritten Fetalmonat produzierten Zellen gehört. In der intrazellulären Globinsynthese wo die den Aufbau der Aminosäureketten regulierenden Faktoren dafür ausschlaggebend sind ob fetales oder bleibendes Hämoglobin entsteht ist gewissermassen mit der Hämatopoese ein Schritt in reiferer Richtung genommen worden. Da Erythrozyten mit bleibendem Hämoglobin auch bei ganz jungen Feten gefunden worden sind darf man annehmen dass der für die Bildung des bleibenden Hämoglobins verantwortliche Mechanismus schon gleich zu Beginn der Fetalentwicklung existiert wennschon seine Aktivität anfänglich geringer ist als in den letzten Wochen der Fetalentwicklung Es ist offenbar so dass derjenige Teil der Hämoglobinsynthese der in den Primitivzellen abnimmt in eine andere Gruppe übergeht auf Zellen die gewissermassen die Stufe von den Primitivzellen zu reiferen Formen darstellen In dieser hämatopoetischen Übergangsperiode steht die Hämoglobinsynthese sozusagen am Scheideweg Reine Zellen die nur bleibendes Hämoglobin enthalten werden nur wenige gefunden. Sobald die Hämatopoese im hepatolienalen System reger wird steigt auch der Anteil des bleibenden Hämoglobins es treten aber noch keine reinen bleibendes Hämoglobin enthaltenden Zellen auf sondern der Blutfarbstoff verteilt sich auf Mischzellen. Da wir noch im Frühstadium der Fetalentwicklung stehen kommen die Mischzellen der Gruppe II zustande die vorwiegend Fetalhämoglobin führen

Andererseits ist es nicht ausgeschlossen dass in diese Gruppe II aus der Primitivphase herstammende und bloss Fetalhämoglobin enthaltende Erythrozyten geraten sind die schon in Degeneration begriffen sind und auf die Elutionsfärbung so reagieren dass das Hämoglobin sich als an gefällte Flocken färbt. Da jedoch die Streuung bei beiden nicht so gross ist dass sie die statistische Signifikanz beeinträchtigte darf man wohl zu dem Endresultat

Tabelle Die Z hlen der au serhalb der Quart le bleibenden Erythrozyten bei de monatige Feten

Gruppe		Gruppe II		Gruppe III		Gruppe IV		Gruppe	
	Zahl		Zahl		Zahl		Zahl		Zahl
10		48	2	[illegible]		3	6	—	—
9		4		48	4	—	—	—	—
6		46	4	42	2	—	—	—	—
—	—	4		40	1	—	—	—	—

kommen dass in dem von mir angewandten Elutionsfarbeverfahren die Zellen sich bei den viermonatigen Feten in der von der Kurve angezeigten Weise verteilen

Zellen der Gruppe I Bei den Zellen der Gruppe I ist die Streuung relativ gering Die Extremwerte verteilen sich bei den übrigen Zellgruppen folgendermassen

M	I	II	III	IV	V
1	10	40	43	5	<1
	10	42	43	5	<1
3	9	42 %	44	5	<1
	9	40	4	4	<1
5	6	46	44	3	<1
6	5	43	40	4	<1
	5	46	40	3	<1
8	5 %	40	42	5	<1
9	5	51	4	4	<1

Die Obergrenze liegt bei 10 %. Der Abstand zur Untergrenze der Primitivzellen der dreimonatigen Feten 13 % ist so gross dass die Differenz in der Frequenz der Primitivzellen zwischen diesen beiden Monaten auch statistisch signifikant ist. Im Lauf des vierten Monats wiederum ist in der Zellverteilung der Extremwertfälle der Primitivzellen keine ausgeprägte Übereinstimmung wahrzunehmen. In den Gruppen III und IV sind die den Maximal- und Minimalwerten der Primitivzellen entsprechenden Werte durchweg ungleichmässig Die Veränderungen der Primitivzellen spiegeln sich zuvörderst in den Zellen der Gruppe II wider

Zellen der Gruppe II In dieser Gruppe ist die Streuung schon grösser als bei den Zellen der Gruppe I Die Zellverteilung der Extremwerte ist wie folgt

M	I	II	III	IV	V
1		48	42 %	3	<1
2	5	50	4	5	<1 %
3	8	4	40 %	5	<1
4		47	43	3	<1 %
5		48	44	3	<1
6	6	46	47	5	<1
	5 %	46	44	5	<1
8	5	48	46	3 %	<1
9	10	40 %	45	5	<1
10	9	40	47	4	<1
11	8	40	40	4	<1 %
12	8	40	4 %	5	<1
13		40	40	5	<1 %
14		50	48	5	<1 %
15	6	40 %	40 %	4	<1

In den Extremwertfällen der Gruppe II ist das Auftreten der anderen Zellgruppen ganz uneinheitlich und die Veränderungen in der Gruppe II reflektieren sich ziemlich gleichmässig in allen Zellgruppen.

Zellen der Gruppe III Der Medianwert 45 % fällt genau in die Mitte der Streuung, während die Maximal- und Minimalwerte 50 % und 40 % sind. Die Zellverteilung der ausserhalb des Quartilgebiets liegenden Extremwertfälle ist in den einzelnen Gruppen völlig uneinheitlich. Die Differenzwerte sehen folgendermassen aus:

M	I	II	III	IV	V
1	6	40	40 %	4 %	<1
2	6	40 %	40 %	4 %	<1
3	8	40	40 %	4 %	<1
4	7	40 %	40 %	5 %	<1 %
5	7	40	48	5 %	<1
6	6	41 %	40 %	5 %	<1
7	5	48	42 %	5	<1
8	7	40	42 %	3	<1

Zellen der Gruppe II In dieser Gruppe ist die Streuung wieder gering Das obere Quartil bildet zugleich den Extremwert. Die Zellverteilung in den Extremwertfällen ist folgende

V	I	II	III	IV	V
1	8	4 %	44	3	< 1 %
	%	46	44	3	< 1 %
3	7 %	47	43	3	< 1 %
4	%	43	4	3	< 1
	6	44	4	3	< 1 %
6	5	46	46	3	< 1

Die Verteilung ist gleichmässig und die Werte bleiben in allen Zellgruppen innerhalb des Quartilbereichs.

Bei der *Zellgruppe* I sind auch hier keine registrierbaren Schwankungen wahrzunehmen

Fünfmonatige Feten.

Die Kurve die die Zellverteilung bei fünfmonatigen Feten veranschaulicht (Diagramm 8) verläuft wieder so wie in den anderen Fetalmonaten mit Ausnahme des vierten Monats. Aus dieser Kurve ist ersichtlich dass die Primitivzellen weiterhin stark zurückgehen. Der Anteil der Zellen der Gruppe II ist noch ziemlich hoch Medianwert 28 %, aber die Abnahme im Vergleich zum vorigen Monat ist als Medianwert ausgedrückt immerhin 16 %. Die Minderung der Zellen sowohl der Gruppe I wie der Gruppe II ist der Gruppe III zugute gekommen deren Medianwert nun 64 beträgt. Dank dessen hat die Kurve nun wieder einen spitzen Gipfel. Die Streuung in Gruppe II und III ist die bisher grösste Aus der Streuungstabelle (Tabelle 3) sind die ausserhalb der Quartile liegenden Extremwerte der einzelnen Zellgruppen ersichtlich.

Tabelle 3 Die Zahlen der ausserhalb der Quartile liegenden Erythrozyten bei den fünfmonatigen Feten

Gruppe I		Gruppe II		Gruppe III		Gruppe IV		Gruppe	
	Zahl		Zahl		Zahl		Zahl	%	Zahl
4	3	40	1	68	2	8	1	—	—
1	3	35	1	62		6	1	—	—
—	—	33	2	60	3	3	3	—	—
—	—	32	1	45	1	—	—	—	—
—	—	25	3	—	—	—	—	—	—

Zellgruppe I Den Extremwerten gemäss verteilen die Zellen sich folgendermassen.

V	I	II	III	IV	V
1	4	30	60	6 %	< 1
	4	30 %	62 %	4 %	< 1 %
3	4	78	64	4	< 1
4	1	40 %	53 %	4 %	< 1 %
5	1	33 %	63 %	3 %	< 1 %
6	1	35	60	4 %	< 1 %

Die Frequenzschwankungen der Primitivzellen verteilen sich ziemlich gleichmässig auf die übrigen Zellengruppen. Fall 4 bildet allein im Vergleich zu den übrigen eine so grosse Ausnahme dass höchstwahrscheinlich entweder eine irrige Deutung oder eine pathologische Erscheinung in Frage steht

Zellgruppe II Streuung ist insbesondere nach oben hin wahrzunehmen und die den Extremwerten entsprechende Zellverteilung sieht folgendermassen aus:

N	I	II	III	IV	V
1	1	40	55	4	< 1
2	1	35	60	4	< 1
3	1	33	63	3	< 1
4	2	33	62	3	< 1
6	3	32	60	5	< 1
	2	25	65	8	< 1
	2	25	68	5	< 1
8	2	5	68	5	< 1

In dieser Gruppierung sind beide Extremwerte auffallig. Der Maximalwert entspricht dem Minimalwert der Zeillen der Gruppe III und die Frequenz der Zellen der Gruppe IV ist 4 o, was der Medianwert und zugleich auch der Unterquartilwert ist. Der Mindestwert der Zellen der Gruppe II entspricht dem Höchstwert in Gruppe IV und auch die Zellen der Gruppe III liegen oberhalb der Medianebene. Bei Fall Nr 1 handelt es sich jedoch um den gleichen Fall, auf dessen eventuelle Fehlerhaftigkeit bereits vorn hingewiesen wurde. In Fall Nr 6 zeigt sich als ein gewisses Vorzeichen der mit dem Fortschreiten der Fetalentwicklung hervortretende Übergang zu reiferen Zellformen, der sich im Lauf der nächsten Monate immer deutlicher geltend macht.

Zellgruppe III. Auch diese Zellen weisen eine beträchtliche Streuung auf, sie ist aber umgekehrt wie bei den Zellen der Gruppe II hier in der Hauptsache abwärts gerichtet. Die Zellverteilung in den Extremwertfällen ist folgende

N	I	II	III	IV	V
1	2	25	68	6	< 1
2	2	25	68	5	< 1
3	2	33	62	3	< 1
4	4	30	62	4	< 1
5	4	30	60	6	< 1
6	3	32	60	5	< 1
	1	35	60	4	< 1
8	1	40	55	4	< 1

In der Streuung tritt keinerlei Regelmässigkeit zutage. Fall 8 ist auch hier wieder derjenige, dessen Zuverlässigkeit suspekt ist. In der Gruppe II und III könnte dieser Fall freilich zu den viermonatigen Feten passen, aber die niedrige Frequenz der Primitivzellen passt doch zuvörderst in die Gruppe der fünfmonatigen.

Zellgruppe IV. Die Streuung ist fast ebenso gering wie in der Gruppe der Primitivzellen. In den Extremwertfällen verteilen die Zellen sich folgendermassen.

N	I	II	III	IV	V
1	2	25	65	8	< 1
2	4	30	60	6	< 1
3	3	28	66	3	< 1
4	2	33	62	3	< 1
5	1	33	63	3	< 1

Auch in diesen Fallen bleiben die Werte der übrigen Zellgruppen innerhalb des Quartilbereichs. Vergleicht man z.B. Fall 1 und Fall 4 miteinander, so sieht man klar, wie uneinheitlich die Frequenz der anderen Zellgruppen ist.

Zellgruppe V. In dieser Gruppe sind weiterhin keine registrierbaren Veränderungen zu beobachten.

Sechsmonatige Feten.

Aus der Kurve in Diagramm 9 lässt sich zum erstenmal unverkennbar entnehmen, wie der Anteil des bleibenden

3

Hämoglobins allmählich anzusteigen beginnt. Bei den reinen Zellen der Gruppe V lässt sich die Zunahme noch nicht beobachten aber die vorwiegend bleibendes Hämoglobin enthaltenden Mischzellen der Gruppe IV sind um 50 im Vergleich zum vorherigen Monat gestiegen

Die Streuungstabelle 4 gibt die Anzahl der Extremwertfälle in den einzelnen Zellgruppen an.

Tabelle 4 Die Zahlen der ausserhalb der Quartil bleibenden Erythrocyten bei den se kunonat gen Feten

Gruppe I		Gruppe II		Gruppe III		Gruppe IV		Gruppe V	
	Zahl		Zahl		Zahl	%	Zahl		Zahl
5	1	40	1	66	2	9	2	1	3
4	1	33	2	65	2	8	2	—	—
—	—	28	3	64	2	4	4	—	—
—	—	27	1	59	2	—	—	—	—
—	—	26	2	58	1	—	—	—	—
—	—	—	—	53	1	—	—	—	—

Zellgruppe I Die Frequenz der Primitivzellen ist ungefähr die gleiche wie im vorausgegangenen Fetalmonat, und die Streuung ist gering Ausserhalb des Quartilbereichs bleiben nur zwei Fälle.

N	I	II	III	IV	V
1	6 %	29 %	40 %	8 %	< 1
2	4 %	29	63 %	4	< 1

Die Verteilung auf die Zellgruppen liegt in Nähe der Medianebene

Zellgruppe II Die Streuung ist ebenso gross wie im vorigen Fetalmonat. Die Verteilung auf die anderen Zellgruppen ist in den Extremwertfällen folgende

N	I	II	III	IV	V
1	2	40	53	5	< 1
2	2	33	59	6	< 1
3	1	31	9		< 1
4	1	28	62	9	< 1
	2	8	60	9	1
6	3 %	8	61	5	< 1
	3	2	64	6	< 1
8	1	6	64		< 1
9	1	26	66 %	6	1

Auffallend ist der untere Pol wo niedrigen Werten der Gruppe II hohe Werte der Gruppen III und IV entsprechen Die Zellen der Gruppe III liegen in den Fällen 6—9 alle oberhalb vom Oberquartil aber in dem zur gleichen Klasse zählenden Fall 5 ist die Frequenz der Zellen der Gruppe III 60 % was das Gleiche ist wie die Unterquartilzahl. Bei den Zellen der Gruppe IV tritt in den Extremwertfällen 4—9 Schwankung zwischen dem Höchstwert 9 % und dem Unterquartil 5 % auf Also auch in dieser Gruppe ist keinerlei statistische Übereinstimmung festzustellen

Zellgruppe III Die Streuung dieser Zellen ist gross die Differenz zwischen den Extremwerten beträgt 11 %. Die übrigen Zellgruppen verteilen sich in den Extremwerten folgendermassen.

N	I	II	III	IV	V
1	1 %	26 %	66 %		< 1
2	1 %	26	66 %	%	< 1
3	1 %	29 %	65 %	5 %	< 1
4	2 %	29	65 %	4 %	< 1
5	3 %	28	64 %	5	< 1 %
6	3 %	27	64 %	6 %	< 1
7	1 %	33 %	59 %	7 %	< 1 %
8	2 %	33 %	59 %	6 %	< 1
9	2 %	32 %	58 %	7 %	1 %
10	2 %	40 %	53 %	5	< 1

Der Mindestwert entspricht dem Höchstwert in der Gruppe II Diese Probe könnte bezüglich dieser beiden Zellgruppen wieder für einen vier monatigen Fetus passen aber die niedrige Prozentzahl der Primitivzellen ist trotzdem eher für die sechsmonatigen charakteristisch. Bei Gruppe IV reflektieren sich die Schwankungen der Gruppe III ganz uneinheitlich. Zwischen Gruppe III und II lässt sich insofern eine Übereinstimmung nachweisen als den ausserhalb des oberen Quartils der Gruppe III liegenden Werten die im unteren Quartil der Gruppe II oder unterhalb davon liegenden Werte entsprechen und bei den Minimalwerten der Gruppe III ist die Situation der Gruppe II gegenuber genau umgekehrt.

Zellgruppe IV Die Streuung ist relativ gering Die Extremwerte gruppieren sich folgendermassen.

Nr	I	II	III	IV	V
1	1 %	28 %	62	9	< 1
2	2 %	28	60	9	1
3	1 %	30 %	60	8	< 1
4	2 %	30 %	60	8	< 1
5	2 %	29 %	65	4	< 1
6	3	32	61	4	< 1
7	3 %	30	63	4	< 1
8	4	29 %	63	4	< 1

Hier wird der schon bei Gruppe II festgestellte Sachverhalt bestätigt, dass die direkte Beziehung der Minimalwerte der Gruppe II zur hohen Frequenz der Gruppe IV nur scheinbar ist.

Zellgruppe V Hier treten zum erstenmal drei Fälle auf bei denen die Frequenz der Zellen von Gruppe V über den vagen Mindestwert gestiegen ist allerdings nur auf 1 %.

Hier ist die Zellverteilung folgende

Nr	I	II	III	IV	V
1	1 %	30 %	60	8	< 1
	%	32	58 %		1
3	2 %	8	60	9	1 %

Bezüglich der Primitivzellen tritt keine Abweichung vom Normalniveau auf. Die Zellen der Gruppe III liegen im Bereich des unteren Quartils. Die Zellen der Gruppe IV haben eine ausgesprochene Tendenz zu höheren Werten. Dies steht nicht im Einklang damit, dass bei den sechsmonatigen Feten sich schon Zunahme des reiferen Hämoglobins geltend macht.

Siebenmonatige Feten.

Aus der Kurve in Diagramm 10 ist ersichtlich, dass in diesen Fetalmonat der Frequenzgipfel der nur Fetalhämoglobin enthaltenden Zellen der Gruppe III also fallt. Der Gipfel ist zuvörderst dank der Zellen der Gruppe II zustandegekommen. Die Primitivzellen sind im Vergleich zum vorherigen Monat zuruckgegangen, Gruppe IV ist unverändert geblieben. Zum ersten mal treten auch Zellen der Gruppe V so zahlreich auf dass sich ein Medianwert von 1 / ergibt.

Hämatopoetisch stehen wir wieder in einer gewissen Kulmination, denn das Knochenmark beginnt jetzt in ständig wachsendem Masse für die Blutbildung zu sorgen. Dies kommt an der Peripherie als Zunahme der reiferen Zellformen zum Ausdruck.

Die Tabelle 5 gibt die Anzahl der Extremwerte an

Tabelle 5. Die Zahlen der ausserhalb der Quartile bleibenden Erythrozyten bei den siebenmonatigen Feten

Gruppe I		Gruppe II		Gruppe III		Gruppe IV		Gruppe V	
	Zahl		Zahl		Zahl		Zahl		Zahl
		2		4	1		4		
—	—	19	1		1	—	—	—	—
—	—	—	—	65	—	—	—	—	—

In den hier zur Rede stehenden Fetalmonat fällt die eigenartige Situation, dass die Primitivzellen und die ausschliesslich bleibendes Hämoglobin enthaltenden Zellen einander das Gleichgewicht halten. Ferner ist auch die Streuung gering und ungefähr gleich gross. Auch im allgemeinen ist die Streuung in den Proben dieses Monats klein, was sich schon in der Anzahl der Streufälle zeigt. Weil die Streuung gering ist und ausserhalb der Quartilbereiche nur wenig Extremwerte liegen, habe ich alle Extremwertgruppen in die gleiche Tabelle genommen.

N	I	II	III	IV	V
Gruppe I					
1	2	0			1
	2	19		8	1
Gruppe II					
3	0	15	65	5	1
4	1	[illegible]	65	4	1
		19	0	8	1
Gruppe III					
6		2	4		1
	1	31	2	6	1
8	1	4	65		1
9	0	2	65	5	1
Gruppe IV					
10	2	19	0	8	1
11	1	24	0	8	1
12	1	20	0	8	1
13	1	3	6	8	1
Gruppe V					
14		22	2	4	2
15	1	2	0	5	2

Da in dieser Phase die Blutbildung zur wachsenden Produktion reiferer Zellformen übergeht, ist es interessant zu sehen, wie diejenigen Fälle, in denen Primitivzellen maximal vorkommen, sich in diese Reifungstendenz einfügen. Nr. 1 und 2 repräsentieren derartige Fälle. Bei den Zellen der Gruppe V weicht die Frequenz nicht von der Medianebene ab. Der Anteil der Zellen der Gruppe IV kommt dem Maximum nahe. Gruppe III liegt in der Medianebene und Gruppe II in den untersten Werten. Diese Extremwertfälle befolgen die allgemeine Richtung, welche die Mediankurve angibt.

Achtmonatige Feten

Aus der Kurve in Diagramm 11 ist ersichtlich, dass die Medianebene der Primitivzellen jetzt 0 / ist. Die Zellen der Gruppe II sind im grossen und ganzen im Vergleich zum vorherigen Monat gleich geblieben. Der Medianwert der Frequenz der Zellen von Gruppe III ist auf 65 / gefallen. Diese Abnahme reflektiert sich als Anstieg der Gruppen IV und V, und diese Wandlung ist eine direkte Folge der hämatopoetischen Reifung.

Die Streuungstabelle 6 gibt die Anzahl der Extremwerte an.

Tabelle 6. Die Zahlen der [au]ßerhalb der Quartile bleibenden Erythrozyten bei den achtmonatigen Feten

Gruppe I		Gruppe II		Gruppe III		Gruppe IV		Gruppe V	
	Zahl		Zahl		Zahl		Zahl		Zahl
1	2	5	1	68	1	18	1	5	1
—	—	25	1	6	3	15	5	3	2
—	—	23	1	65	2	11	2	—	—
—	—	22	1	62	2	—	—	—	—
—	—	18	1	62	1	—	—	—	—
—	—	—	—	64		—	—	—	—

Zellgruppe I. Der Medianwert dieser Primitivzellen ist 0%. Nur in 2 von 30 Fällen kamen Primitivzellen in 1% vor. Die Verteilung dieser Fälle auf die anderen Zellgruppen ist folgende:

V	I	II	III	IV	V
1	1	20	65	12	
2	1	21	65	13	1

Diese zwei Fälle könnten hinsichtlich Gruppe I und V in den vorherigen Fetalmonat passen, aber die anderen Werte sind doch charakteristisch für den achten Fetalmonat.

Zellgruppe II. Streuung macht sich zuvörderst von der Medianebene aufwärts geltend. Die Zellverteilung der Extremfälle ist folgende:

V	I	II	III	IV	V
1	0	25	60	13 ♀	2
2	0	5	62	12 ♀	
3	0 ♂	23	62	14	1
4	0 ♀	22	65	12	2
5	0	18	6 ♂	13	2
6	0 ♂♀	18	65	13	1

Die Maximalwerte der Gruppe II entsprechen den Minimalwerten der Gruppe III. Sonst sind keine Übereinstimmungen wahrzunehmen.

Zellgruppe III. Die Streuung ist hauptsächlich nach unten gerichtet. Die Extremwerte entfallen auf die Zellgruppen folgendermassen:

V	I	II	III	IV	V
1	0	19	68	11 ♂	2
	0	18	6	13	2
3	0	8	6	1	1
5	0 ♀	19	6	11	3
5	0	0	65	15	1
6	0	22	65	12	
	0 ♀	21	63	14	2 ♀
8	0	21	63	15	2
9	0	23	62	14	1
10	0	20	60	18	2
11	0	25	60	13	2

In Fall 10 ist das Gegengewicht zum Minimalwert der Gruppe III der Maximalwert der Gruppe IV und der Oberquartilwert der Gruppe V. Dieser Sachverhalt steht im keinem Widerspruch zur Gesamtentwicklung.

Zellgruppe IV. Die Streuung ist ziemlich beschränkt. Die Verteilung auf die Zellgruppen den Extremwerten gemäss ist folgende:

V	I	II	III	IV	V
1	0	20	64	18	2
2	0	20	65	18 ♀	1 ♂
3	0	19 ♂	63	13	1 ♀
4	0	19	63	15	1 ♂♀
5	0 ♂	19	65	15	1 ♀
6	0	18	64	15	1 ♂
7	0	19	67	11 ♂	8
8	0 ♂	19	68	11 ♀	2 ♀

Fall 1 ist der gleiche Fall 10 in der vorigen Tabelle und er weist also auf Reifung der Fetalentwicklung hin. Bei zwei Minimalwerten liegen die Zellen der Gruppe V oberhalb der Medianebene, sie weisen aber trotzdem keinen deutlichen Unterschied auf.

Zellgruppe V Die Streuung ist ausserordentlich klein. Extremwertfälle gibt es nur drei und ihre Zellverteilung ist folgende:

N	I	II	III	IV	V
1	0 %	19 %	65 %	12	4
2	0 %	0	66	11	3
3	0	19	67	11	3

Die Verteilung auf die Zellgruppen bleibt eng bei der Medianebene und ergänzt gewissermassen die Gesamtkurve.

Neunmonatige Feten

Aus der Kurve in Diagramm 12 geht hervor dass die Primitivzellen bei Null liegen, der Medianwert der Zellgruppe II ist weiterhin 20 %, aber die Frequenz der Gruppe III sinkt ständig und beträgt jetzt 59 %. Entsprechend steigt der Anteil der Zellgruppen IV und V Die Reifung in der Hämatopoese nimmt somit auch an der Peripherie immer klarere Formen an.

Aus der Tabelle 7 ist wiederum die Anzahl der Extremwerte ersichtlich

Tabelle 7 Die Zahlen der ausserhalb der Quartile bleibenden Erythrozyten bei den neunmonatigen Feten

Gruppe		Gruppe II		Gruppe III		Gruppe IV		Gruppe V	
	Zahl		Zahl		Zahl		Zahl		Zahl
—	—	25	1	63	1	0	2		1
—	—	18	5	6	4	18	2	4	4
—	—	—	—	5	6	—	—	—	—
—	—	—	—	54	1	—	—	—	—

Zellgruppe I Diese Primitivzellen wurden in keiner einzigen Probe gesehen.

Zellgruppe II Streuung tritt zuvörderst oberhalb der Medianebene auf Die Zellverteilung der Extremwertfälle war folgende:

N	I	II	III	IV	V
1	0 %	25 %	50	20	5 %
2	0	18	59 %	17 %	6 %
3	0	18 %	63	15	4
4	0 %	18	60	17	5 %
5	0 %	18	58	20 %	4 %
6	0	18	58	18 %	6 %

In Fall 1 ist der Höchstwert der Zellen der Gruppe II 25 %, und die Zellen der Gruppe III fallen auf 50 %. Diese Frequenz der Gruppe III nämlich 50 % differiert von der allgemeinen Skala so weitgehend dass sie vermutlich von einem Fetus stammt der trotz der strengen Auswahl irgendwie pathologisch war Für diesen Verdacht spricht der Umstand, dass die Probe ganz vereinzelt dasteht und von den anderen keinerlei Stütze erhält. Um einen Deutungsirrtum dürfte es sich schwerlich handeln, weil ganz klar ausgeprägte Zellen der Gruppe III in Frage stehen. Hinsichtlich der Mindestwerte zeigen sich in der Verteilung der anderen Zellgruppen keine wesentlichen Schwankungen.

Zellgruppe III Die Streuung tritt in ziemlich weitem Gebiet auf Die Zellverteilung dieser Extremwertfälle ist folgende:

Wesentliche Abweichungen lassen sich in der Zellgruppierung nicht beobachten. Fall 12 ist der gleiche der bei Gruppe II bereits besprochen wurde

N	I	II	III	IV	V
1	0	18	63	15	4
	0	0	62	16	4
3	0	20	6	16	4
4	0	18	62	15	
5	0	20	6	1	6
6	0	19	5	1	
	0	21	5	16	6
8	0	21	8	1	3
9	0	1	3	1	5
10	0	0	8	1	4
11	0	20	8	19	4
12		2	60	20	5

Zellgruppe II Die zahlenmässig grösste Streuung ist um das Quartilgebiet konzentriert, so dass nur wenige regelrechte Extremwerte vorkommen. Diese Extremwerte entfallen auf die Zellgruppen folgendermassen

N	I	II	III	IV	V
1		25	50	2	5
	0	18	58	2	4
3	0	18	58	18	6
4	0	0	50	10	4
5	0	19	58	18	3

Die Frequenzschwankungen der Zellen der Gruppe IV verteilen sich gleichmässig auf die anderen Zellgruppen. Fall 1 wurde schon in den vorigen Gruppen besprochen.

Zellgruppe I In dieser Gruppe ist die Streuung sehr klein Die Zellverteilung der Extremwerte ist folgende:

N	I	II	III	IV	V
1	0	19	5	17	
	0	20	60	16	4
3		19	60	17	4
4	0	20	50	10	4
5	0	20	60	16	4

Hier sind zuvörderst die Gruppen IV und V interessant. Den Minimalwerten der Gruppe V entsprechen Maximalwerte der Gruppe IV Dies steht im Widerspruch zu der Tatsache dass die Zellen der Gruppen IV und V hauptsächlich das bleibende Hämoglobin enthalten, das die vor der Entwicklungsreife stehenden Feten besitzen. Da die hämatopoetische Entwicklung in dem Stadium steht wo Mischzellen der Gruppe IV noch reichlicher vorkommen sind entsprechend die ausschliesslich bleibendes Hämoglobin enthaltenden Zellen der Gruppe V *weniger vertreten aber die Ge*samtmenge des bleibenden Hämoglobins bleibt unverändert.

Zehnmonatige Feten

Die Kurve im Diagramm 13 veranschaulicht die Situation beim ausgetragenen Neugeborenen. Primitivzellen waren in den Proben keine zu sehen. Bezüglich der Zellen der Gruppe II ist eine leichte Abnahme im Vergleich zum vorherigen Monat eingetreten. Die gleiche sinkende Tendenz macht sich auch bei den Zellen der Gruppe III geltend Gruppe IV hat sich stark vermehrt die Zunahme in der Medianebene ist dem vorherigen Monat gegenüber 50 . Am ausgeprägtesten und bedeutsamsten ist der Anstieg jedoch bei Gruppe V Der Medianwert ist doppelt so hoch wie der entsprechende Wert im Monat vorher Gruppe IV und V verdeutlichen hämatopoetisch den Fortschritt der in der Gesamtentwicklung der Frucht stattgefunden hat.

Die Tabelle 8 zeigt die numerische Streuung und die Verteilung auf die Zellgruppen in den Extremwertfällen

Tabelle 8 Die Zahlen der ausserhalb der Quartile bleibenden Erythrozyten bei den sechsmonatigen Feten

Gruppe I		Gruppe II		Gruppe III		Gruppe IV		Gruppe V	
%	Zahl	%	Zahl	%	Zahl	%	Zahl	%	Zahl
—	—	5	1	60	2	28	3	19	4
—	—	—	—	50	3	16	2	18	3
—	—	—	—	—	—	—	—	16	2
—	—	—	—	—	—	—	—	15	
—	—	—	—	—	—	—	—	8	2

Zellgruppe I Primitivzellen wurden in den Proben keine gefunden

Zellgruppe II In dieser Gruppe bleibt ausserhalb des Unterquartils nur ein einziger Fall Seine Zellverteilung ist folgende

N	I	II	III	IV	V
1	0	5	60	16	19

Charakteristisch ist hier eine grosse Gruppe V und auch Gruppe III repräsentiert einen Maximalwert während wiederum Gruppe II und IV niedrige Werte haben Die Streuung nach oben ist so klein dass der Maximalwert 14 beträgt während der Medianwert 12 % ist. Unten sind einige von diesen 14 Fällen in Zellengruppen eingeteilt

N	I	II	III	IV	V
1	0	14	5	23	11
2	0	14	52	23	11
3	0	14	52	22	12
4	0	14	52	24	10
5	0 %	14	40	24	12

Die Zellverteilung bleibt im grossen und ganzen in der Nähe der Mediankurve

Zellgruppe III Streuung hauptsächlich nach unten. Die Zellverteilung in den Extremwertfällen ist folgende

N	I	II	III	IV	V
1	0	5	60	16	19
2		10	60	21	9
3	0	10	40	25	16
4	0	10	50	28	12
5	0	11	40	28 %	11
6	0	13	50	18	19
	0	14	50	24	12

Abgesehen von den Zellen der Gruppe III ist die Verteilung sehr uneinheitlich. Bei den Gruppen IV und V lässt sich wieder feststellen dass hohen Werten der Gruppe IV im allgemeinen niedrige in der Gruppe V entsprechen und umgekehrt.

Zellgruppe IV Auch für diese Gruppe ist es charakteristisch dass das Hauptgewicht der numerischen Streuung sich in engster Nähe der Medianebene konzentriert Eigentliche Streuung tritt nur nach unten hin auf Die Zellverteilung der Extremwerte ist folgende

N	I	II	III	IV	V
1	0	10	50	28	12
2		11	50	28	11
3	0	11	52	28	9
4	0	5	60	18	19
5	0	10	55	16	19

Den Minimalwerten entsprechen hohe Werte der Gruppe V und bei den Maximalwerten sind die Zellen der Gruppe IV in der Medianebene.

Zellgruppe V. Die Streuung der Zellen der Gruppe V ist sehr interessant. Sie ist sowohl numerisch wie prozentual nach oben hin am grössten. In den Extremwertfällen ist die Zellverteilung folgende:

N	I	II	III	IV	V
1	0	5	60	16	19
2	0 %	10 %	5	16	19
3	0	10	1	0	19
4	0	12	50	18	19
5	0	10	52	20	18
6	0	10 %	54	18	18
	0	10	55	1	18
8	0	10	50	4	16
9	0 %	10	55	19	16
10	0 %	10 %	55	20	15
11	0 %	10	54	21	15
12	0	11	5	26	8
13	0	12 %	5 %	23	8

In den Maximalwertfällen betrifft die Minderung der anderen Zellgruppen in erster Linie Gruppe II und IV m.a.W. also Zellen die teilweise bleibendes Hämoglobin enthalten. Wir haben hier wiederum ein Zeichen von der hämatopoetischen Reifung, infolgederen der Anteil der ausschliesslich bleibendes Hämoglobin enthaltenden Zellen zunimmt und die Mischzellen entsprechend abnehmen.

BESPRECHUNG DER ERGEBNISSE

Das Hauptziel der vorliegenden Untersuchung ist es gewesen mit Hilfe der hier angewandten Farbemethode die Frequenzverhältnisse der fetales und bleibendes Hämoglobin enthaltenden Erythrozyten in den verschiedenen Stadien der Fetalentwicklung aufzudecken und so den Boden für die Untersuchungen zu schaffen die auf die Identifizierung der oftmals bei obstetrischen pathologischen Zuständen im mutterlichen Kreislauf anzutreffenden fetales Hämoglobin enthaltenden Erythrozyten hinzielen. Ferner ist untersucht worden welche Möglichkeiten es gibt die Resultate für die Bestimmung des Entwicklungsalters der Frucht anzuwenden

Betrachtet man nun das Hauptproblem der Fragestellung so ist es am zweckmassigsten dass man zunächst die grössere Komponente also die fetalhamoglobinhaltigen Erythrozyten ins Auge fasst. Das fetale Hamoglobin an sich sein Wesen und seine Eigenschaften sind schon so allgemein bekannt dass sie keine Diskussion mehr hervorrufen, aber die Frage ob das fetale Hämoglobin als eine Ganzheit aufzufassen ist oder ob sich ein hinsichtlich seiner Eigenschaften differierendes *primitives* Fetalhämoglobin unterscheiden lässt findet schon ihre Anhänger und ihre Gegner Drescher und Künzer (1954) haben mit der Alkalidenaturation bei einem unter 12 Wochen alten Fetus ein drittes Hämoglobin gefunden das eine andere Denaturationsgeschwindigkeit hat als das bleibende und das Fetalhamoglobin. Butler (1960) fand bei der Agar Elektrophorese wo als »Träger« 1,5 %iges Agar-Gel diente und pH 6 0—6 5 war bei einem 3,5 cm langen 9 Wochen alten Fetus ein Hämoglobin das mit anderer Geschwindigkeit wanderte als das Erwachsenen und das Fetalhämoglobin. Auch Halbrecht & Klibanski (1956) sowie Zilliacus (1960) sind bei der Papierelektrophorese zu dem Resultat gekommen dass es ein spezielles primitives Hämoglobin geben muss das hauptsächlich in den ersten Fetalwochen auftritt. Betke seinerseits nimmt zu dieser Frage keine endgültige Stellung, und zu ganz negativem Resultat sind Matsuda und Schroeder u. Mitarb (1960) gekom

men. Während im Untersuchungsgut von Drescher Künzer Halbrecht und Klibanski sowie Zilliacus das Alter der Feten zwischen 7 und 12 Wochen schwankte befand sich im Material von Matsuda u. Mitarb nur ein einziger 7 Wochen alter Fetus und die meisten waren 15 Wochen alt oder älter

Betrachtet man Diagramm 1 meines Materials so sieht man dass die Frequenz der von mir als primitive Erythrozyten bezeichneten Zellen in den ersten 3—5 Fetalmonaten steil abfällt. Aus der Hämatopoese wissen wir dass die Kurve der mesoblastischen Periode einen weitgehend gleichen Verlauf nimmt Wenn man das in diesen primitiven Erythrozyten enthaltene Hämoglobin untersuchen will muss dies begreiflicherweise zu einem Zeitpunkt geschehen wo diese Primitivzellen am zahlreichsten auftreten m.a.W. eben dann wenn das mesoblastische System für die Hämatopoese sorgt. Der Hauptteil des Untersuchungsguts von Matsuda u. Mitarb liegt ausserhalb dieser Periode und hieraus erklärt sich auch das negative Resultat zu dem sie gekommen sind. Aufgrund meiner eigenen Untersuchungsergebnisse vermag ich nichts Sicheres darüber zu sagen was das in diesen primitiven Erythrozyten enthaltene Hämoglobin ist. Wir haben gesehen, dass es sich zur Elutionsfarbung genauso verhalt wie das fetale Hämoglobin überhaupt. Das morphologisch von den anderen roten Blutzellen abweichende primitive Gefüge dieser Erythrozyten ihre Grösse Durchmesser im Mittel 11—12 μ die plumpe Struktur und die Kernhaltigkeit sowie der Umstand dass ihr Vorkommen hauptsachlich in die mesoblastische Periode fällt, rechtfertigt die Bezeichnung als besonders primitive Erythrozyten

Diese Bezeichnung, *primitive Erythrozyten* fusst in erster Linie auf der Färbbarkeit dieser Zellen m.a.W. darauf dass das in ihnen enthaltene Hämoglobin sich nicht im sauren Puffer auflöst sondern in der Zelle zurückbleibt weshalb die Zelle sich so farbt wie auch die anderen fetalhämoglobinhaltigen Zellen. Man kann sie daher nicht zu den *Normoblasten* rechnen weil zu den Normoblasten auch Zellen gehören die bleibendes Hämoglobin enthalten

Diese primitiven Erythrozyten fallen in der hamatopoetischen Einteilung welche die Erythrozytenbildung in eine megaloblastische und eine normoblastische Periode einteilt zunächst in die megaloblastische Periode wobei die den Hauptteil ausmachenden kernhaltigen Zellen den Megaloblasten entsprechen während die kleine Menge kernloser Primitivzellen ihrerseits den Megalozyten entspricht aber ganz offenbar gehören auch kernhaltige Normoblasten zu dieser Gruppe der Primitivzellen.

Im Hinblick auf all dies lasst sich sagen dass meine Ergebnisse am ehesten mit denjenigen Untersuchungen im Einklang zu stehen scheinen die für die Existenz eines Primitivhämoglobins sprechen

In Diagramm 1 beginnt die Kurve beim dritten Fetalmonat und zu diesem das Alter von drei Fetalmonaten anzeigenden Punkt gehören alle 13

Woche alten und jüngeren Feten. Die Frequenz der primitiven Erythrozyten ist an diesem Punkt 10 %. Beim vierten Monat, wo die mesoblastische Periode als abgeschlossen angesehen werden darf, beträgt die entsprechende Prozentzahl 7, von welchem Wert sie steil auf 1—2 % absinkt. Nach dem *siebenten Fetalmonat verläuft die Kurve bereits bei Null.* Wenn wir nun daran festhalten, dass die Primitiverythrozyten hauptsächlich in der mesoblastischen Periode produziert werden, wie ist es dann zu erklären, dass noch so lange wie durchschnittlich drei Monate nach Abschluss der mesoblastischen Periode diese Zellen in den gefärbten Präparaten zu sehen sind? Der Umstand, dass die Grenze zwischen den verschiedenen Orten der Blutbildung nicht scharf ist, gibt die Erklärung dafür, dass in der Frequenz der Primitivzellen der steile Abfall erst im fünften Fetalmonat einsetzt. Die Einteilung der Hämatopoese in drei verschiedene Perioden führt leicht zu dem Gedanken, dass diese primitiven Zellen ausschliesslich im Dottersack gebildet wurden, die anderen fetalhämoglobinhaltigen Zellen entsprechend im hepatolienalen System und die bleibendes Hämoglobin enthaltenden Zellen aus*schliesslich im Knochenmark. Eine so* scharfe Grenze entspricht jedoch nicht den wirklichen Verhältnissen, denn wir haben in Proben von der Leber eines neunwöchigen Fetus sowohl primitive Erythrozyten gefunden wie auch solche, die mit aller Wahrscheinlichkeit bleibendes Hämoglobin enthielten. Walker & Turnbull (1955) haben mit dem Alkalidenaturationsverfahren in der Blutprobe von einem 13 Wochen alten Fetus bleibendes Hämoglobin gefunden. Thomas u. Mitarb. (1960) haben mit radioaktiv markiertem Glycin in der Leber wie im Knochenmark sowohl fetales als auch bleibendes Hämoglobin nachgewiesen. Betke und Kleihauer (1958) ihrerseits fanden mit der Elu*tionsfärbemethode Fetalhämoglobin im* Knochenmark.

Ganz offenbar werden weitaus die meisten primitiven Erythrozyten eben im mesoblastischen System gebildet, während wiederum die hauptsächliche HbF-Produktion im hepatolienalen System stattfindet und die HbA-Bildung im Knochenmark. In der Übergangsperiode sind die Grenzen der Zellbildung in den verschiedenen Systemen jedoch dann wahrscheinlich ziemlich diffus. Fernerhin muss man auch bedenken, dass die in den gefärbten Präparaten sichtbaren Zellen verschiedenes Alter haben, und obwohl die Lebensdauer der fetalen Erythrozyten kürzer ist als die der Erwachsenenerythrozyten, beträgt sie im Durchschnitt doch immerhin 70 Tage (Vest 1962, Foconi & Sjölin 1959, Erlandsson et al. 1958). Man darf daher mit gutem Recht annehmen, dass die Produktion der primitiven Erythrozyten, *auch wenn sie in der Hauptsache* schon im Lauf des vierten Fetalmonats beendigt wäre, doch in geringerem Masse eventuell auch noch in den zwei folgenden Monaten in vereinzelten Herden in der Leber und im Knochenmark weiterginge. Die meisten von den in den Präparaten aus dem sechsten und siebenten Fetalmonat sichtbaren primitiven Erythrozyten dürften jedoch

degenerierende Primitivzellen sein, die aus dem vierten und fünften Fetalmonat stammen.

Die als *Gruppe II und III* unterschiedenen Mischzellen werden zusammen besprochen, denn hinsichtlich ihrer Grundeigenschaften sind sie weitgehend einander identisch. Der Unterschied liegt einzig und allein im Mengenverhältnis der in ihnen enthaltenen Hämoglobine. Meine eigenen Beobachtungen stehen gut im Einklang mit dem von Kleihauer und Betke (1961) ausgesprochenen Gedanken, dass fetales und bleibendes Hämoglobin zusammen in ein und derselben Zelle auftreten können. Ähnliche Beobachtungen haben auch Hoffbauer (1962) und Braun (1962) gemacht.

Die Resultate, die verschiedene Forscher mit Alkalidenaturationsmessungen vom Mengenverhältnis des bleibenden und des fetalen Hämoglobins in den verschiedenen Stadien der Fetalentwicklung erhalten haben, sind nicht von der gleichen Grössenklasse wie die von mir mit der Farbemethode erzielten Werte. Vom fünften bis zum achten Fetalmonat sind die Werte des bleibenden Hämoglobins bei Messung mit Alkalidenaturation in allen Untersuchungen etwa 10 %, während wiederum in meinem Material die Frequenz der ausschliesslich bleibendes Hämoglobin enthaltenden Erythrozyten in der entsprechenden Zeit nur > 1 % beträgt. Beim ausgetragenen Fetus variieren die Alkalidenaturationswerte zwischen 20 und 40 %, und in meinem Material ist der entsprechende Medianwert der Zellgruppe V 11 % (Extremwerte 8 und 19 %).

Zum Vergleich habe ich Alkalidenaturationsmessungen nach der Methode von Chernoff-Singer bei Feten im Alter von 7, 9 und 10 Monaten durchgeführt und Blutproben von den gleichen Feten wurden gleichzeitig nach dem Elutionsfärbeverfahren untersucht.

Monate alter Fetus

Im gefärbten Präparat verteilten die Zellen sich folgendermassen.

I	II	III	IV	V
1			6	1

die entsprechende Alkalidenaturation ergab folgende Werte

HbF 92 HbA 8

9 Monate alter Fetus

Gefärbtes Präparat

I	II	III	IV	V
	19	60	15	6

Alkalidenaturation.

HbF 86 HbA 14

10 Monate alter Fetus

Gefärbtes Präparat.

I	II	III	IV	V
[illegible]	12 %	5	25	11

Alkalidenaturation.

HbF [illegible] % HbA 21,8

Das Verhältnis ist bei der Alkalidenaturation das gleiche wie auch in den anderen Materialien. Bei dem siebenmonatigen Fetus ist von dem bleibenden Hämoglobin in den Zellen der Gruppe V nur 1/8 enthalten, alles übrige verteilt sich auf die Mischzellen der

Gruppen II und IV, wobei die unreifen Zellen der Gruppe II numerisch den grössten Teil ausmachen. Bei den neun- und zehnmonatigen Feten ist in den Zellen der Gruppe V schon etwa die Hälfte des bleibenden Hämoglobins enthalten und entsprechend ist auch der Anteil der vorwiegend bleibendes Hämoglobin enthaltenden Mischzellen der Gruppe IV stark angestiegen. Diese Resultate stehen im Einklang mit der Mischzellentheorie von Kleihauer und Betke.

Aus Diagramm 2 ist ersichtlich, dass der Frequenzgipfel der vorwiegend Fetalhämoglobin enthaltenden Mischzellen in den vierten Fetalmonat fällt und dann mit dem Fortgang der Fetalentwicklung ziemlich steil absinkt. Entsprechend beginnt in der Kurve 4 der steile Anstieg erst nach dem siebten Fetalmonat. Dieser ganz entgegengesetzte Verlauf der zwei Kurven veranschaulicht schön die Unterschiede zwischen diesen Mischzellen.

Die HbF — HbA Erythrozyten gehören noch zur unreifen Form der Zellentwicklung und das Hauptgewicht ihres Vorkommens fällt in den Anfang der Fetalentwicklung, während wiederum die HbA + HbF Erythrozyten schon die Periode repräsentieren, in welcher die Bildung der roten Blutzellen hauptsächlich im Knochenmark vor sich geht und der Anteil des bleibenden Hämoglobins ausschlaggebend zu steigen beginnt.

Die Frage, ob diese Zellen der Gruppe IV irgendetwas mit den Zellen zu tun haben, die Kleihauer für fetalhämoglobinhaltige Retikulozyten hält, drängt sich auf, wenn man ihre Frequenzkurve in Diagramm 4 betrachtet und sie mit der Kurve der bleibendes Hämoglobin enthaltenden Zellen in Diagramm 5 vergleicht. Der Anstieg beginnt in beiden Kurven an der gleichen Stelle und auch der Anstiegswinkel ist ungefähr der gleiche. Handelt es sich hier um eine Situation, welche die Beziehung zwischen den reifen Erythrozyten und den im gleichen Verhältnis zunehmenden Retikulozyten zum Ausdruck bringt, oder ist das analoge Auftreten der von mir als HbA + HbF Mischzellen bezeichneten Zellen nur eine Folge davon, dass ihre Menge in eben dem Verhältnis zunimmt wie die Menge der ausschliesslich bleibendes Hämoglobin enthaltenden Zellen? Die grösste Frequenz dieser Mischzellen der Gruppe IV fällt, wie aus Diagramm 4 hervorgeht, ins letzte Drittel der Fetalentwicklung, d.h. hämatopoetisch in die medulläre Periode.

Kleihauer konnte in Präparaten vom Nabelschnurblut des ausgetragenen Fetus, die mit einer speziellen Retikulozytenfärbung behandelt worden waren, zwei Zelltypen unterscheiden, von denen der eine die normalen Retikulozyten des Menschen darstellte, während Kleihauer den anderen für fetalhämoglobinhaltige Retikulozyten ansah.

In meinem Untersuchungsgut stellen die bleibendes Hämoglobin enthaltenden Retikulozyten kein Problem dar, weil der Blutfarbstoff sich in ihnen bei der Elutionsfärbemethode auflöst, die Zellen nur als leere Hüllen zu sehen sind und keine Netzstruktur zum Vorschein kommt. Es wäre natürlich denkbar, dass manche von

diesen Zellen der Gruppe IV tatsächlich fetalhämoglobinhaltige Retikulozyten wären, und dass es sich bei dem rot angefärbten Teil dieser Mischzellen den ich für ungelöst gebliebenes Fetalhämoglobin gehalten habe in Wirklichkeit um Netzwerk handle Als aber die Proben die derartige Zellen enthielten, in solcher Färbung behandelt wurden welche die für die Retikulozyten charakteristische Struktur zur Darstellung bringt, wurden nach Art der Retikulozyten gefärbte Zellen nur 1—2 erhalten Da ausserdem nach Senon (195) ein beträchtlicher Teil von den Zellen die sich in gleicher Weise wie die Retikulozyten färben garkeine Jugendformen der Erythrozyten sondern umgekehrt Degenerationsformen sind, in denen sich koagulierte basophile Substanz anfärbt und eine Retikulozytenstruktur vortäuscht wäre es nicht ausgeschlossen dass die Zellen der Gruppe IV die auf die Spezialfärbung wie die Retikulozyten reagieren eben solche degenerierte Fetalerythrozyten waren m.a.W beispielsweise Zellen der Gruppe IV die aus einer früheren Entwicklungsstufe stammen als die in welcher die Probe genommen wurde und in der Degeneration soweit fortgeschritten sind dass sie sich in der von Senon beschriebenen Weise färben.

Die ausschliesslich Fetalhämoglobin enthaltenden Erythrozyten der *Gruppe III* stellen die am leichtesten zu deutende Gruppe dar weil die Zellen sich intensiv rot färben und daher im Praparat klar zu erkennen sind. Die Frequenzkurve dieser Zellen im Lauf der Fetalentwicklung (Diagramm 3) hat die gleiche Richtung wie die Kurve die das Auftreten des Fetalhämoglobins in den verschiedenen Fetalmonaten dem Alkalidenaturationsverfahren gemäss darstellt. Die Ähnlichkeit des Gesamtverlaufs ist eine Folge davon, dass der grösste Teil des Fetalhämoglobins sich eben in diesen Zellen der Gruppe III befindet, da aber fetaler Blutfarbstoff auch in den Mischzellen enthalten ist fallen die Werte dieser reinen HbF Zellen logischerweise niedriger aus als die mit der Alkalidenaturation erzielten Gesamtwerte des Fetalhämoglobins In der sonst ziemlich gleichmässigen Kurve bildet der vierte Fetalmonat eine schroffe Abweichung, denn die Frequenz der HbF Zellen fällt an diesem Punkt von 62—64 auf 45 %. Vergleichspunkte zu diesem Absteigen gibt es in den anderen Untersuchungen keine Was mag nun diese eben in den vierten Fetalmonat fallende steile Abnahme in der Frequenz der HbF Zellen herbeiführen? Theoretisch kann man davon ausgehen dass gerade in diesem Stadium der Übergang von der mesoblastischen zur hepatolienalen Periode stattfindet. Man könnte sich denken dass zu diesem Zeitpunkt zwischen den verschiedenen Blutbildungssystemen eine Art Grenzrevision vor sich geht wobei allerdings das Hauptgewicht noch auf der primitiven Seite liegt. Die vorwiegend Fetalhämoglobin enthaltenden Mischzellen sind gewissermassen auch Grenzrevisionszellen. Sie bilden sozusagen eine Stufe zwischen den primitiven und den reiferen Erythrozyten stehen aber selber den primitiven Formen näher Aus diesem Grunde stehen sie auch zahlen

Nachdem wir nun die im Lauf der Fetalentwicklung auftretenden Erythrozyten in fünf verschiedene Gruppen klassifiziert ihre gegenseitigen Unterschiede und zugleich auch ihre individuellen Eigenschaften bestimmt sowie ferner festgestellt haben in welchem Umfange die Frequenz jeder Zellgruppe im Lauf der Fetalentwicklung schwankt können wir durch Vergleich dieser Resultate mit den eventuell im mütterlichen Kreislauf anzutreffenden fetalhämoglobinhaltigen Erythrozyten nachweisen ob die letzteren von der Mutter oder vom Fetus stammen. Dies ist besonders in Fällen bedeutsam wo Verdacht auf eine Blutung aus dem Fetus in die Mutter besteht Sofern dann im mütterlichen Kreislauf diese für den Fetus charakteristischen Zellen gefunden werden hat der Obstetriker einen direkten Fingerzeig für die Wahl der therapeutischen Massnahmen.

Die *zweite Komponente der Fragestellung* war die Untersuchung der Möglichkeiten die das Material als Grundlage für die Bestimmung des tatsächlichen Entwicklungsalters der Frucht bieten könnte

Gerbie (1959) und Mitarb sind mit der Alkalidenaturationsmodifikation von Singer der gleichen Frage nachgegangen Sie haben zum Ausgangspunkt die Fetalhamoglobinmengen genommen und untersuchten welche diesbezüglichen Unterschiede zwischen Fruhgeburten ausgetragenen Fruchten und übertragenen Kindern bestehen In ihrem Material waren 50 um zwei Wochen zu fruh geborene Kinder enthalten bei denen der durchschnittliche Fetalhämoglobingehalt 80 7 / betrug ferner 120 ausgetragene Kinder mit einem entsprechenden Hämoglobingehalt von 73 3 sowie 25 um zwei Wochen übertragene Neugeborene bei denen die durchschnittliche Fetalhämoglobinmenge 67 6 , war Die Differenzen zwischen diesen Gruppen sind den Autoren gemäss auch statistisch signifikant. Brody (1960) hat sein Material von 200 Neugeborenen nach dem Gewicht in drei Gruppen eingeteilt. I = unter 2 500 g II = 2 500—3 999 g und III = über 4 000 g schwere Kinder In der Gruppe I erhielt er einen Fetalhämoglobingehalt von 89 66 /o, in Gruppe II 77 39 /o und in Gruppe III 74 40 /o. Statistisch ist die Differenz zwischen der ersten und der zweiten Gruppe signifikant während sich wiederum zwischen der zweiten und dritten Gruppe keine beweiskraftigen Unterschiede nachweisen liessen. Beide Untersuchungen haben gemeinsam dass die Fetalhämoglobinmengen in den zwei letzten Wochen der Fetalentwicklung und entsprechend bei zunehmendem Gewicht kleiner werden, in beiden besteht zwischen den Fruhgeburten und den ausgetragenen Kindern ein signifikanter Unterschied aber in Bezug auf die Übertragung ist der Sachverhalt nicht so klar obwohl die Tendenz die gleiche ist.

In meinem Material wurde die Bestimmung des Fetalalters zunächst aufgrund der Frequenzverhaltnisse der verschiedenen Erythrozytentypen angestrebt. Das Fetalhämoglobin ist ja auf drei verschiedene Zellgruppen verteilt weshalb ein direkter Vergleich mit den quantitativen Ergebnissen

nicht möglich ist. Das Gleiche gilt auch für das bleibende Hämoglobin

Aus dem für die einzelnen Fetalmonate ausgearbeiteten Diagramm geht hervor dass man aufgrund aller fünf Gruppen nicht direkt das Fetalalter bestimmen kann weil im Ganzen zwischen den verschiedenen Gruppen keine signifikante Gesetzmässigkeit festzustellen ist.

Stellt man nun die Frequenzen der ausschliesslich Fetalhämoglobin (HbF) enthaltenden Erythrozyten in den drei letzten Fetalmonaten zusammen erhält man folgende Tabelle

Tabelle 11 Frequenz der HbF Erythrozyten (Gruppe III) in den drei letzten Fetalmonaten

Entwicklungsalter	Medianwert	[illegible]	[illegible]	Zahl
8 Monate	6 [illegible]	64 6[illegible]	68 60	36
9 Monate	59	60 50	63 50	2
10 Monate	[illegible]	[illegible] 2	60 50	85

Die Differenz zwischen dem achten und dem zehnten Monat ist signifikant. Wenn man entsprechend die ausschliesslich bleibendes Hämoglobin (HbA) enthaltenden Erythrozyten zusammenstellt bekommt man Tabelle 12.

Die Differenzen zwischen diesen drei Monaten sind ebenfalls statistisch signifikant. Korreliert man diese zwei Gruppen miteinander so kann man sagen, ob der Fetus ausgetragen oder um vier oder eventuell acht Wochen zu früh geboren ist.

Tabelle 12 Frequenz der HbA Erythrozyten (Gruppe I) in den drei letzten Fetalmonaten

Entwicklungsalter	Medianwert	[illegible]	[illegible]	Zahl
8 Monat	1	2 1	4 1	36
9 Monate	5	6 5	[illegible]6	2
1[illegible] Monat	11	12 1	19 0	85

In meinem Material befanden sich auch 9 Neugeborene deren Geburtsgewicht mehr als 4 500 g betrug Bei diesen ist der Medianwert der ausschliesslich bleibendes Hämoglobin enthaltenden Erythrozyten 18 [illegible] (19 /16 [illegible]) Das Material ist klein aber die Differenz zum entsprechenden Medianwert der Erwachsenen 11 (12 /10 [illegible]) ist jedenfalls wennschon nicht signifikant doch mindestens so beträchtlich dass sie Anlass zu weiteren Untersuchungen gibt. Ferner ist es bemerkenswert da s diese Beobachtung weitgehend mit den Resultaten übereinstimmt die Gerbie bei der Gruppierung seines Materials nach dem Gewicht erzielt hat wobei zwischen den Gruppen 2 500 g 3 000 g und über 4 000 g ein statistisch signifikanter Unterschied bestand.

Eine Gruppe für sich stellen bei der Bestimmung des Fetalalters die primitiven Erythrozyten (HbFp-Zellen) dar In Normalfällen werden sie in den letzten drei Fetalmonaten nicht mehr angetroffen Gewisse pathologische Zustände wie z.B die Erythroblastose machen eine Ausnahme. Die Fetalmonate 5—6— bilden bezüglich der Frequenz der Primitivzellen eine gleich-

mässige Periode Es handelt sich offenbar um die Degenerationsphase dieser Zellen in welcher die Produktion neuer Zellen ganz minimal ist Hinsichtlich der drei letzten Fetalmonate ist der Sachverhalt klar Primitivzellen kommen dann normalerweise nicht mehr vor Wenn bei einem anamnestisch sieben Monate alten Fetus mehr als 2 Primitivzellen gefunden werden liegt der Verdacht nahe dass entweder die anamnestische Angabe nicht stimmt oder dass ein pathologischer Zustand in Frage steht.

Die Bestimmung der Frequenz der Primitivzellen könnte vielleicht auch bei Mehrlingsschwangerschaften bedeutsam sein, wo man aufgrund ihres Vorhandenseins oder Fehlens das Entwicklungsalter der Feten beurteilen kann das nicht immer mit Gewicht und Länge im Einklang steht. Auch bei gewöhnlichen Frühgeburten kann man nach dem gleichen Prinzip Anhaltspunkte für die Prognose des Neugeborenen erhalten Es muss in diesem Zusammenhang noch ausdrücklich darauf hingewiesen werden, dass mein Material ausgelesen ist und diese Auswahl kommt natürlich am deutlichsten eben in den letzten Schwangerschaftswochen zum Vorschein. Es wurden nur gesunde Neugeborene mitgenommen bei denen auch im intrauterinen Leben wenigstens keine registrierbaren Störungen wahrzunehmen waren. Thomas u. Mitarb (1960) haben nachgewiesen dass bei Asphyxie also Hypoxämie der Frucht das Verhältnis zwischen bleibendem und fetalem Hämoglobin zugunsten des letzteren verändert wird. Langfristige Asphyxie der Frucht z.B in Fällen von Plazentarinsuffizienz kann somit dazu führen dass der Fetalhämoglobingehalt des Fetus noch bei der Geburt höher als normalerweise ist. Da mein Material ein Bild von der Normalsituation vermitteln soll, geht daraus nicht hervor wie weitgehende Veränderungen die verschiedenen pathologischen Zustände wie Toxämie vorzeitige Lösung der Plazenta sowie funktionelle Störungen der Plazenta im Verhältnis von HbA und HbF herbeiführen. Wenn diese Frage einmal aufgeklärt sein wird und die Resultate mit diesem Normalmaterial korreliert werden, lässt sich die Maturität mit dem hier angewandten Färbeverfahren vielleicht noch genauer bestimmen.

SCHLUSSFOLGERUNGEN

Mit Hilfe der von Kleihauer und Betke entwickelten Elutionsfärbemethode sind in gefärbten Präparaten von fetalen Blutproben fünf Erythrozytentypen unterschieden worden, die sich unterschiedlich zu dieser Färbung verhalten.

Gruppe I (Primitive Erythrozyten). Diese Zellen differenzieren auch morphologisch deutlich von den übrigen Erythrozytengruppen. Sie sind grösser, haben einen mittleren Durchmesser von 11—12 μ und besitzen grösstenteils einen Kern. Nur etwa 10 von diesen primitiven Zellen sind kernlos und sie dürften wohl den von Mundorff als *Megalozyten* bezeichneten roten Blutzellen entsprechen.

Das in diesen Zellen enthaltene Hämoglobin löst sich im Puffer nicht auf. Sie werden hauptsächlich in den drei ersten Fetalmonaten angetroffen, kommen aber in kleiner Menge auch noch beim siebenmonatigen Fetus vor. Danach sind diese Zellen in den Präparaten nicht mehr gefunden worden.

Gruppe II (Mischzellen mit vorwiegend Fetalhämoglobin). Diese Zellen stellen schon kernlose Normoblasten dar, aber das in ihnen enthaltene Hämoglobin ist nicht homogen. Der grösste Teil des Hämoglobins ist im Puffer unlösliches Fetalhämoglobin, das sich rot als dünne Fädchen anfärbt, während das in geringerer Menge vorhandene sog. bleibende Hämoglobin sich auflöst und die entsprechenden Stellen im Präparat farblos bleiben. Auch bei diesen Zellen fällt der Frequenzgipfel in das Anfangsstadium der Fetalentwicklung, wonach die Frequenz gleichmässig abfällt.

Gruppe III (Ausschliesslich Fetalhämoglobin enthaltende *Erythrozyten*). Kernlose Erythrozyten, deren ganzes Hämoglobin ungelöst bleibt, weshalb sie sich gleichmässig rot färben. Diese Zellen stellen in allen Fetalmonaten die grösste Gruppe dar.

Gruppe IV (Mischzellen, die vorwiegend bleibendes Hämoglobin enthalten). Kernlose Zellen, in denen der grösste

Teil des Hämoglobins im Puffer lösliches bleibendes Hämoglobin ist. In geringerer Menge enthalten sie Fetalhämoglobin, das sich nicht auflöst und in den Präparaten als rosafarbener Mantel oder schmale Streife zu sehen ist. Der Frequenzgipfel fällt in das letzte Drittel der Fetalentwicklung.

Gruppe V (Ausschliesslich bleibendes Hämoglobin enthaltende Erythrozyten). Diese Zellen sind im gefärbten Präparat als leere Hüllen zu sehen. Das in ihnen enthaltene Hämoglobin hat sich in dem Zitronensäure-Phosphat-Puffer aufgelöst. Die Zellen sind kernlos und entsprechen reifen Erythrozyten. Sie kommen in meinem Material in allen Präparaten vor. Der kleinste Fetus, in dessen aus der Leber genommener Blutprobe im gefärbten Präparat diese farblos gebliebenen bleibendes Hämoglobin enthaltenden Erythrozyten gefunden wurden, war neun Wochen alt und 2,3 cm lang.

Die Frequenz ist bis zum Alter von acht Fetalmonaten ≤ 1 %, wonach sie steil bis zum Medianwert des Erwachsenen 11 % ansteigt.

Da in allen Phasen der Fetalentwicklung sowohl fetales wie bleibendes Hämoglobin enthaltende Zellen vorkommen, in denen diese Hämoglobine entweder allein oder gleichzeitig zusammen auftreten, darf man annehmen, dass die Kraft, von welcher es abhängig ist, wie die Aminosäureketten in der Globinsynthese sich anordnen, m.a.W. welches Hämoglobin jeweils zustandekommt, schon vom Beginn der hämatopoetischen Entwicklung an wirksam ist. Der Unterschied liegt wahrscheinlich darin, dass die Lokalisation der Hämatopoese im Laufe der Fetalentwicklung dafür ausschlaggebend ist, welches Hämoglobin jeweils im Vordergrund steht.

Das in der Fragestellung gesteckte Ziel, die Schaffung des Bodens für die obstetrische Untersuchung in den Fällen, wo die Symbiose Fetus-Plazenta-Mutter irgendwie gestört ist, wurde in der Arbeit insofern erreicht, als die im Lauf der Fetalentwicklung auftretenden Erythrozytentypen identifiziert und die Hauptlinien ihrer Frequenzgrenzen kartiert werden konnten, so dass die Möglichkeit gegeben ist, im mütterlichen Kreislauf diese für den Fetus charakteristischen roten Blutzellen zu unterscheiden.

Was die Möglichkeit der *Bestimmung des Fetalalters* aufgrund der erzielten Resultate betrifft, so lässt sich keine Zellanalyse geben, die für jeden Fetalmonat für sich charakteristisch wäre. Gewisse Einzelheiten kann man jedoch auch in diesem Sinne ausnutzen, und dies sogar mit ziemlicher statistischer Verlässlichkeit.

Die primitiven Erythrozyten stellen ein gewisses Kriterium dar, indem ihr Vorkommen in den letzten drei Fetalmonaten entweder gegen die Zuverlässigkeit der anamnestischen Angaben spricht, oder aber als Zeichen eines pathologischen Zustandes beim Fetus aufgefasst werden kann. Die gegenseitigen Frequenzverhältnisse der Zellgruppen III und V im achten, neunten und zehnten Fetalmonat liefern den Anhaltspunkt für die Bestimmung des Fetalalters mit einer Genauigkeit von vier Wochen.

ZUSAMMENFASSUNG

Es wurden Blutproben von 343 Feten verschiedenen Alters genommen von denen der kleinste 2,3 cm lang und 9 Wochen alt und der grösste ein 54 cm langes 5050 g schweres Neugeborenes war. Das Material ist insofern ausgelesen als nur solche Feten mitgenommen wurden, bei denen nichts Pathologisches nachzuweisen war. Diese Auswahl ist natürlich am zuverlässigsten in den letzten Fetalmonaten. Die Proben aus den vier ersten Fetalmonaten stammen von Fällen von Sectio caesarea minor und die übrigen von spontan geborenen lebenden Feten.

Das Ziel der Arbeit war es zu untersuchen wie sich die Erythrozyten in Blutproben von Feten verschiedenen Alters in den nach der Elutions-Färbemethode von Kleihauer und Betke gefärbten Präparaten in den verschiedenen Stadien der Fetalentwicklung zu dieser Färbung verhalten. Als Lösepuffer wurde Zitronensäure-Phosphatlösung mit pH 3,3 benutzt und zur Färbung wurden Hämatoxylin und Erythrosin angewandt. Auf diese Weise konnten fünf verschiedene Erythrozytengruppen unterschieden werden die folgendermassen klassifiziert wurden.

Gruppe I. Kernhaltige *primitive Erythrozyten* die im Anfangsstadium der Hämatopoese gehören und morphologisch am ehesten den Megaloblasten entsprechen. Das Hämoglobin dieser Zellen löst sich im Puffer nicht auf und ist demgemäss fetales Hämoglobin. Diese roten Blutzellen treten hauptsächlich im ersten Drittel der Fetalentwicklung auf, werden aber minimal noch bei siebenmonatigen Feten angetroffen. Danach sind in den gefärbten Präparaten keine Primitiverythrozyten mehr gefunden worden.

Gruppe II. Kernlose, vorwiegend Fetalhämoglobin enthaltende Mischzellen, deren Fetalhämoglobin in den Zellen resistiert, während das sog. bleibende Hämoglobin sich im Puffer auflöst. Im gefärbten Präparat sieht man das Fetalhämoglobin als rot gefärbte wurmförmige Fädchen, die

den grössten Teil der sichtbaren Fläche der Zelle bedecken. Zwischen diesen Fädchen bleiben schmale leere Gebiete in denen das Hämoglobin sich gelöst hat. Der Frequenzgipfel dieser Zellen fällt in die ersten Fetalmonate sie werden aber doch in allen Stadien der Fetalentwicklung angetroffen.

Gruppe III. Kernlose ausschliesslich fetales Hämoglobin enthaltende Erythrozyten. Da alles Hämoglobin dieser Zellen im Puffer unlöslich ist färben sie sich gleichmässig rot. Der grösste Teil von den in den Proben aller Fetalmonate sichtbaren roten Blutzellen gehört zu dieser Gruppe.

Gruppe IV. Kernlose vorwiegend bleibendes Hämoglobin enthaltende Mischzellen in denen das minder reichlich vertretene Fetalhämoglobin in den Zellen zurückbleibt, während der grösste Teil des roten Blutfarbstoffs sich auflöst. Das in den Zellen zurückgebliebene fetale Hämoglobin ist im gefärbten Präparat entweder als dünner rosafarbener Mantel, oder als schmale Streifen zwischen farblosen Gebieten zu sehen. Der Vergleich zwischen den Alkalidenaturationsmessungen und entsprechenden gefärbten Präparaten stützt die auf dem Unterschied der Färbbarkeit fussende Beobachtung, dass in ein und derselben Zelle zugleich fetales und bleibendes Hämoglobin enthalten sein kann.

Gruppe V. Kernlose, ausschliesslich bleibendes Hämoglobin enthaltende Erythrozyten die im gefärbten Präparat als leere Hüllen zu sehen sind weil das ganze darin enthaltene Hämoglobin sich im Puffer gelöst hat. Diese Zellen treten in allen Proben auf vom kleinsten 9 Wochen alten Fetus bis zum grössten ausgetragenen Neugeborenen. Der Umstand, dass sowohl Zellen mit bleibendem wie Zellen mit fetalem Hämoglobin in allen Stadien der Fetalentwicklung vorkommen, rechtfertigt die Annahme dass die Faktoren welche die Hämoglobinsynthese steuern in der Hämatopoese von Anfang an wirksam sind dass ihre momentane Aktivität aber von der Lokalisation der Hämatopoese bestimmt wird so dass der in Entwicklung befindliche Organismus regulieren kann, welche Hämoglobinart jeweils im Vordergrund steht.

Weiterhin ist in der Untersuchung auch verfolgt worden in welcher Weise die oben besprochenen fünf Zellgruppen sich im Lauf der Fetalentwicklung verteilen. Indem die prozentuale Frequenz einer jeden Zellgruppe für jeden Fetalmonat einzeln berechnet wurde konnten Kurven gezeichnet werden aus denen die Frequenzschwankungen jeder Zellgruppe die ganze Fetalentwicklung hindurch zu entnehmen sind. Die Diagramme 1—5 veranschaulichen die Frequenzschwankungen einer jeden Zellgruppe im dritten bis zehnten Fetalmonat. Aus diesen Diagrammen ist ersichtlich wie die Frequenzentwicklung der Gruppen der hämatopoetischen Phase entspricht. Ausserdem wurden die Diagramme 6—13 ausgearbeitet aus denen hervorgeht, in welcher Weise diese fünf Zellgruppen sich gleichzeitig nach den Fetalmonaten verteilen.

Mit diesen Rechenoperationen wurde die Frage zu klären versucht, ob mit Hilfe des angewandten Färbeverfahrens das Entwicklungsalter der Frucht bestimmt werden kann.

Aufgrund aller fünf Zellgruppen direkt kann man kein für jeden Fetalmonat für sich charakteristisches Diagramm zusammenstellen, weil die Differenzen zwischen den verschiedenen Gruppen nicht einheitlich signifikant waren. Aufgrund des Vorkommens der *primitiven Erythrozyten* kann man den Schluss ziehen, dass in den letzten drei Fetalmonaten keine primitiven Erythrozyten auftreten und dass vom fünften bis zum siebten Fetalmonat die Frequenz dieser Zellen normalerweise 5 nicht übersteigt. Aufgrund des Mengenverhältnisses zwischen Zellgruppe II und V lässt sich das Fetalalter auf vier Wochen genau festlegen, und zwar so, dass man sagen kann, ob der Fetus ausgetragen oder vielleicht vier oder acht Wochen zu früh geboren ist. Dies hat seine Bedeutung besonders in Fällen, wo die anamnestischen Angaben im Widerspruch zu Länge und Gewicht der Frucht stehen.

Die Bedeutung der Untersuchungsergebnisse für die klinische Obstetrik liegt in erster Linie darin, dass die Kenntnis der fünf besprochenen Erythrozytentypen Anhaltspunkte liefert, mit deren Hilfe man die entweder im peripheren Kreislauf der Mutter oder in pathologischen vaginalen Blutungen vorkommenden fetalhämoglobinhaltigen Erythrozyten, die möglicherweise vom Fetus herstammen, identifizieren kann.

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Gruppe IV Kernlose, vorwiegend bleibendes Hämoglobin enthaltende Mischzellen in denen das minder reichlich vertretene Fetalhämoglobin in den Zellen zurückbleibt während der grösste Teil des roten Blutfarbstoffs sich auflöst. Das in den Zellen zurückgebliebene fetale Hämoglobin ist im gefärbten Präparat entweder als dünner rosafarbener Mantel oder als schmale Streifen zwischen farblosen Gebieten zu sehen Der Vergleich zwischen den Alkalidenaturationsmessungen und entsprechenden gefärbten Präparaten stützt die auf dem Unterschied der Färbbarkeit fussende Beobachtung dass in ein und derselben Zelle zugleich fetales und bleibendes Hämoglobin enthalten sein kann.

Gruppe V Kernlose ausschliesslich bleibendes Hämoglobin enthaltende Erythrozyten die im gefärbten Präparat als leere Hüllen zu sehen sind weil das ganze darin enthaltene Hämoglobin sich im Puffer gelöst hat. Diese Zellen treten in allen Proben auf vom kleinsten 9 Wochen alten Fetus bis zum grössten ausgetragenen Neugeborenen. Der Umstand dass sowohl Zellen mit bleibendem wie Zellen mit fetalem Hämoglobin in allen Stadien der Fetalentwicklung vorkommen rechtfertigt die Annahme dass die Faktoren welche die Hämoglobinsynthese steuern in der Hämatopoese von Anfang an wirksam sind dass ihre momentane Aktivität aber von der Lokalisation der Hämatopoese bestimmt wird so dass der in Entwicklung befindliche Organismus regulieren kann, welche Hämoglobinart jeweils im Vordergrund steht.

Weiterhin ist in der Untersuchung auch verfolgt worden in welcher Weise die oben besprochenen fünf Zellgruppen sich im Lauf der Fetalentwicklung verteilen. Indem die prozentuale Frequenz einer jeden Zellgruppe für jeden Fetalmonat einzeln berechnet wurde konnten Kurven gezeichnet werden, aus denen die Frequenzschwankungen jeder Zellgruppe die ganze Fetalentwicklung hindurch zu entnehmen sind. Die Diagramme 1—5 veranschaulichen die Frequenzschwankungen einer jeden Zellgruppe im dritten bis zehnten Fetalmonat. Aus diesen Diagrammen ist ersichtlich wie die Frequenzentwicklung der Gruppen der hämatopoetischen Phase entspricht. Ausserdem wurden die Diagramme 6—13 ausgearbeitet, aus denen hervorgeht in welcher Weise diese fünf Zellgruppen sich gleichzeitig nach den Fetalmonaten verteilen.

Group III Non nucleated red cells containing only foetal haemoglobin. Since none of the haemoglobin of these cells dissolves in the buffer solution they stain an even red The majority of the red cells observable in the specimens from all foetal periods belong to this group

Group IV Non nucleated cells with mixed but predominantly adult haemoglobin in which the smaller amount of foetal haemoglobin remains in the cells while the greater part of the haemoglobin dissolves The persisting foetal haemoglobin appears in the stained specimen either as a light mantle or as narrow streaks between large unstained areas. A comparison of the results of measurements made after alkali denaturation and on the corresponding stained specimens confirms the observation made on the basis of th differences in staining i e that in the same cell both foetal and adult haemoglobin may exist simultaneously

Group V Non nucleated red cell containing only adult haemoglobin and appearing in the stained specimens as empty envelopes since all the haemoglobin had dissolved in the buffer solution. These cells occur in all specimens from the smallest 9-week-old foetus to the largest full term infant The fact that cells containing both adult and foetal haemoglobin are present at all stages of foetal development justifies the belief that the factors regulating haemoglobin synthesis are active from the very beginning of haematopoiesis but that the momentary activity of each of them is determined by the localization of the haematopoiesis and so the developing organism may according to need regulate the type of haemoglobin that predominates at any given moment

Besides classification of the red cells into five groups observations were also made as to how these groups are distributed during cell development By calculating the percentage of each group of cells in each foetal month curves were obtained which illustrate the proportion of the different groups of cells during the entire course of foetal development Diagrams 1 to 5 illustrate separately the amounts of each cell group in the third to tenth foetal months These diagrams show how the frequency of the groups varies during the course of foetal development

Diagrams 6 to 13 have been drawn to show the relative distribution of the cell groups in each foetal month.

The aim of these calculations was to investigate whether it is possible to determine the developmental age of the foetus by means of this staining method

It was not possible directly on the basis of all five cell groups to draw a diagram that would characterize each foetal month separately since the differences between the groups were not statistically significant. On the basis of the occurrence of *primitive cell* it can be concluded that these cells are not present during the three last foetal months and that from the fifth to the seventh foetal month their frequency does not exceed 5 per cent in normal cases On the basis of the

ratio between groups III and V we can determine the foetal age with a certainty of four weeks and consequently state whether the foetus is full-term or possibly four or eight weeks premature This is of particular importance when the anamnestic information and the length and weight of the foetus are contradictory

The significance of the results of the present investigation in clinical obstetrics resides principally in the fact that knowing these five types of red cells we have obtained several criteria by means of which we may identify the red cells containing foetal haemoglobin that occur either in the peripheral circulation or in pathological vaginal discharges and which we suspect to originate from the foetus.

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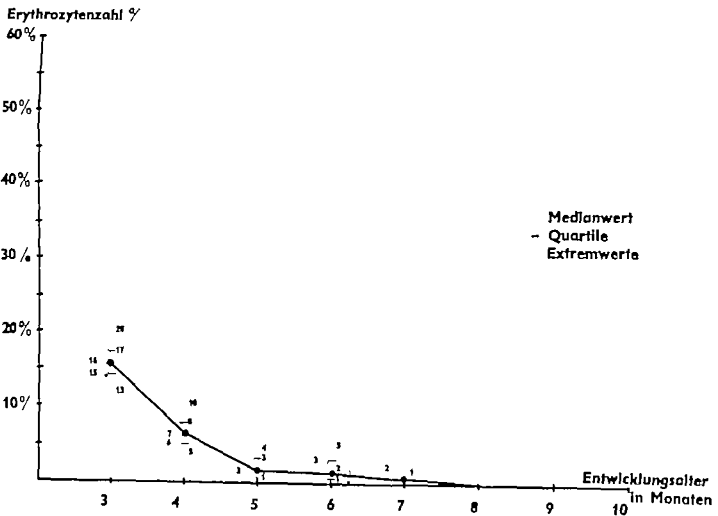
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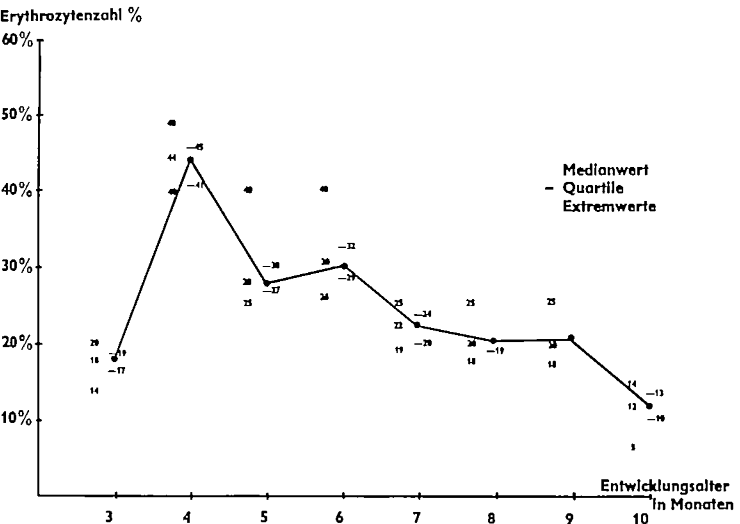
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Di gram 1 Frequen der HbFp-Zellen im La / der Fet lentw kl g


Erythrozytenzahl %
60%
50%
40%
30%
20%
10%
Medianwert
– Quartile
Extremwerte
Entwicklungsalter
in Monaten
3
4
5
6
7
8
9
10

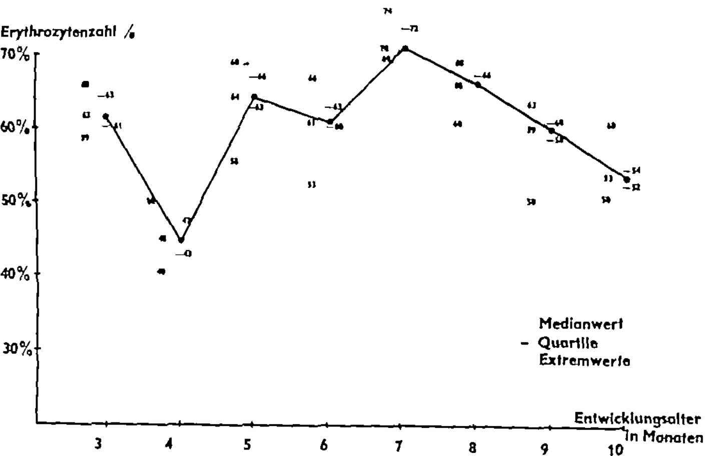


Diagramm 3. Frequenz der HbF-Z[illegible] im La[illegible] der Fetal[illegible]

Erythrozytenzahl %

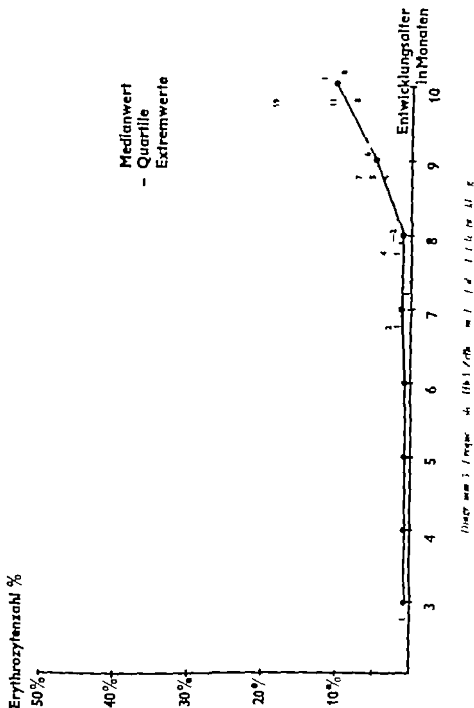
60%
50%
40%
30%
20%
10%

Medianwert
- Quartile
Extremwerte

Entwicklungsalter in Monaten

3 4 5 6 7 8 9 10

Diagramm 4. Frequenz der (HbA + HbF) Z[illegible] im La[illegible] der Fetalentw[illegible]klung



Erythrozytenzahl %
50%
40%
30%
20%
10%
Medianwert
Quartile
Extremwerte
3
4
5
6
7
8
9
10
Entwicklungsalter
in Monaten

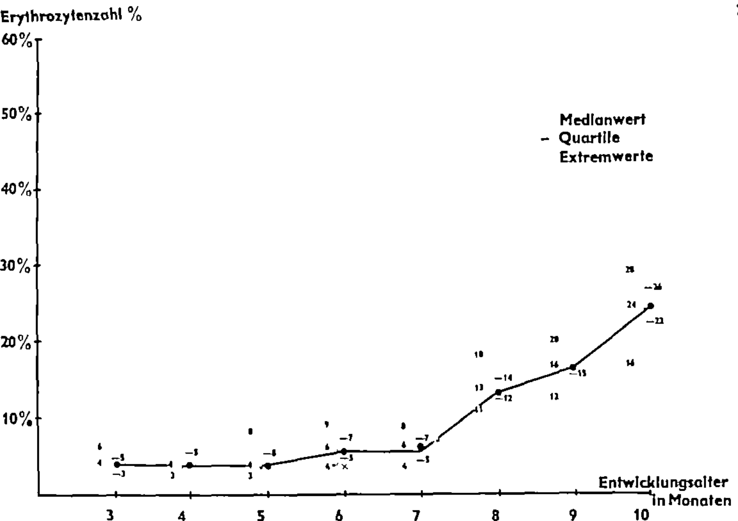


Diagramm 4. Frequenz der (HbA + HbF)-Zellen im Lauf der Fetalentwicklung

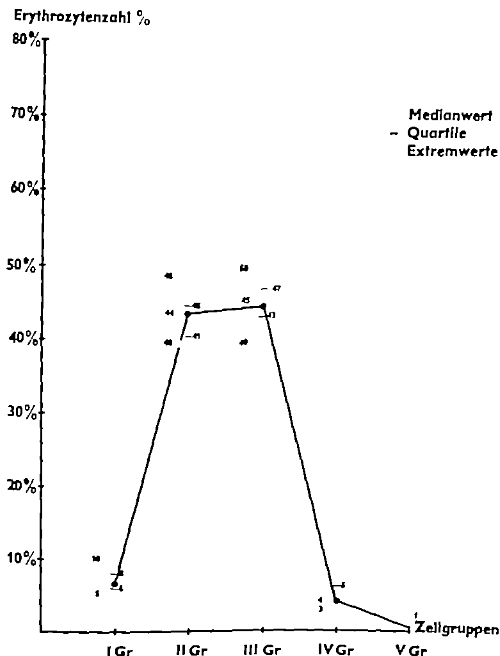
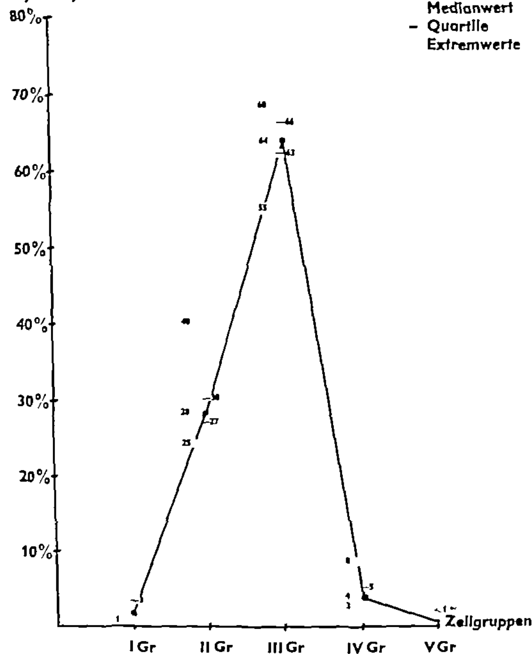


Diagramm 7. Verteilung [illegible] Zellgruppen bei de[illegible] [illegible]monatigen Feten



Di[illegible] gr[illegible] [illegible] auf d[illegible] [illegible] Z[illegible]llgruppen bei d[illegible] [illegible]monatigen F[illegible]

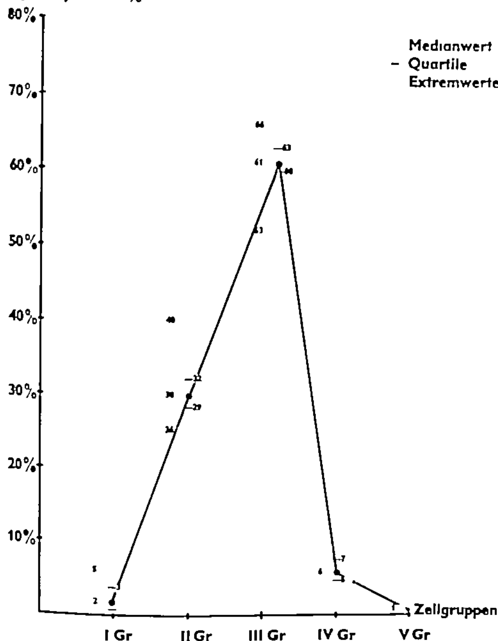


Diagramm 9 Verteilung auf die verschiedenen Zellgruppen bei den sechsmonatigen Feten

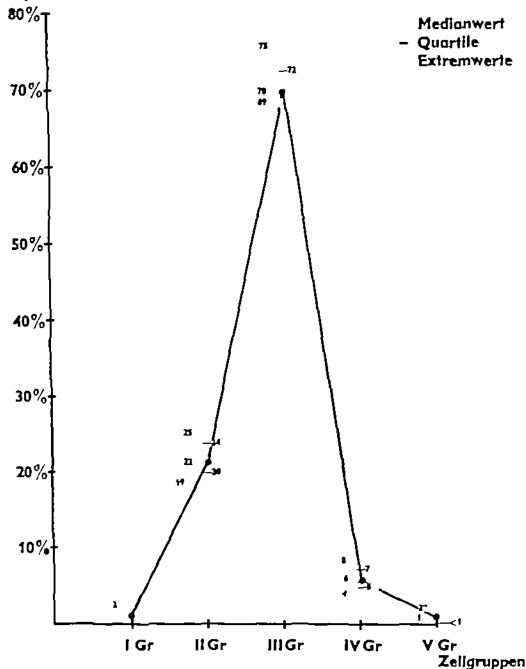


Diagramm 10. [illegible] die [illegible]erschiedenen Zellgruppen bei den siebenmonatigen Feten

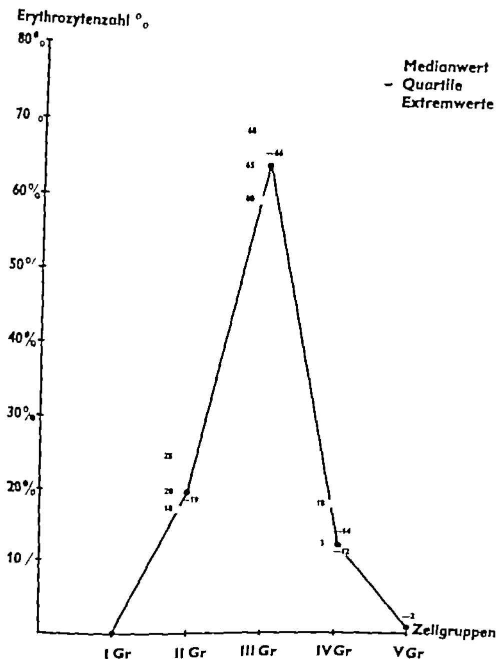


Diagramm 11. Verteilung der verschiedenen Zellgruppen bei den achtmonatigen Feten

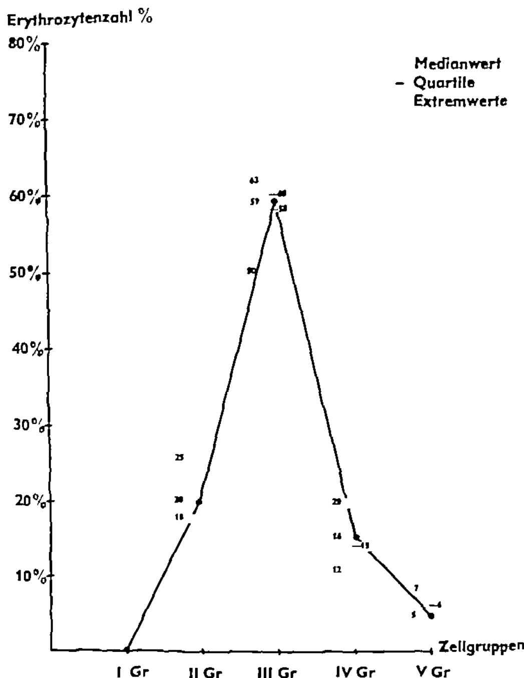


Diagramm 12. Verteilung auf d… …schiedenen Zellgruppen bei den … Fällen.

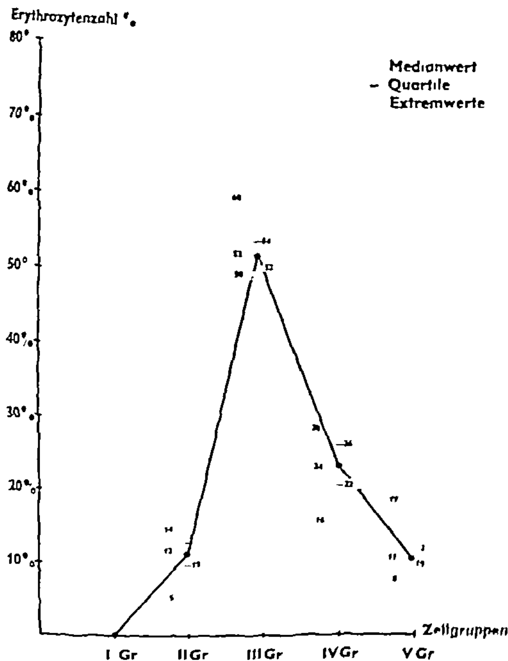

Erythrozytenzahl %
80%
70%
60%
50%
40%
30%
20%
10%
Medianwert
– Quartile
Extremwerte
Zellgruppen
I Gr
II Gr
III Gr
IV Gr
V Gr

Diagramm 13 [illegible]

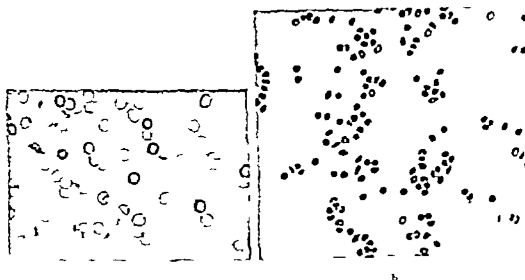
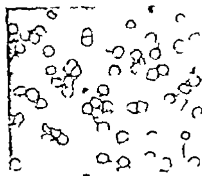
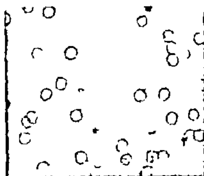


Bild 1
Gefärbtes Präparat [illegible] Gemischs [illegible] 1 Teil Erwachsenenblut und 1 Teil Nabelschnurblut.
b Gefärbtes Präparat [illegible] Neugeborenen [illegible]



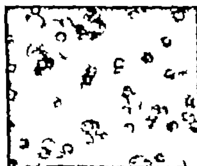
Bild 2
Die Abhängigkeit der Elution [illegible] pH der Puf[illegible] ferlösung. Mischung von Erwachsenen- und N[illegible] belschnurblut im Verhältnis [illegible] pH 3,0 [illegible] Nach Kleihauer
b pH [illegible] pH





b





d

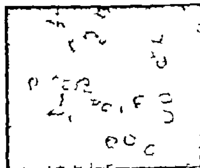


Bild 2.
Einfluss der Konzentration der zur Verdünnung der Blutproben benutzten Kochsalzlösung auf das gefärbte Präparat) 5 %ige NaCl-Lösung. b) 0,9 %ige NaCl-Lösung.) 1 %ige NaCl-Lösung d) 2%ige NaCl-Lösung.) 4 %ige NaCl-Lösung. × 400

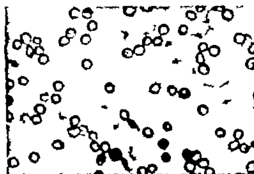


Bild 3

Gefärbtes Präparat der Blutprobe von einem sechsmonatigen Fetus. Man sieht Zellen der Gruppe II sowie auch Zellen der Gruppen I, III, IV und V. × 400

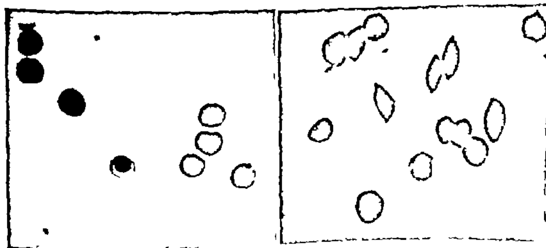
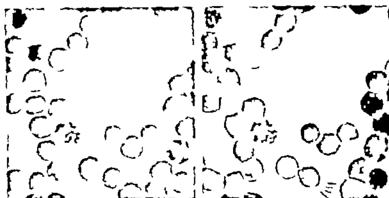


Bild 4

Gefärbtes Präparat der Blutprobe von einem fünfmonatigen Fetus. Man sieht eine kernhaltige Zelle der Gruppe I. × 900

Bild

Gefärbtes Präparat der Blutprobe von einem fünfmonatigen Fetus. Man sieht eine typische Zelle der Gruppe IV. × 900



b

Bild

Retikulozytenpräparat … Nabelschnurblut …) vor und b) die gleiche Stelle nach Elution Zitronensaure Phosphat Puffer pH 3,3

(Nach Kleihauer)



Bild 8

Gefärbtes Präparat von der Leber eines neun-wöchigen Fetus. Man sieht zwei Zellen der Gruppe V sowie ferner noch Primitivzellen und Zellen der Gruppe III. × 480

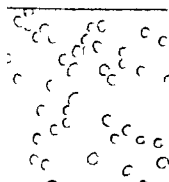


Bild 9
...monatiger Fetus. Man sieht ...
... Zelle der Gruppe V und ... ine
...mitwelle ... × 100

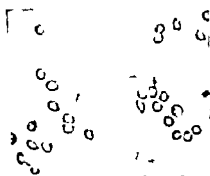


Bild 10
Sechsmonatiger Fetus. Man sieht
eine Zelle der Gruppe V und dar
unter eine Zelle der Gruppe II × 100

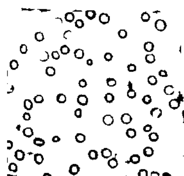


Bild 11
Achtmonatiger Fetus. Man sieht nur
zwei Zellen der Gruppe V und mehrere
Zellen der Gruppe IV ... 100

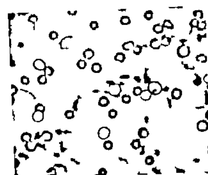


Bild 12.
Zehnmonatiger Fetus. Man sieht
mehrere Zellen der Gruppe V und auch
Zellen der Gruppe IV ... 90

ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 399

PLASMA VITAMIN A AND E IN THE STUDY OF LIPID AND LIPOPROTEIN METABOLISM IN CORONARY HEART DISEASE

BY

RISTO PELKONEN

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PLASMA VITAMIN A AND E IN THE STUDY OF LIPID AND LIPOPROTEIN METABOLISM IN CORONARY HEART DISEASE

From the First Department of Medicine University of Helsinki Finland

PLASMA VITAMIN A AND E IN THE STUDY OF LIPID AND LIPOPROTEIN METABOLISM IN CORONARY HEART DISEASE

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RISTO PELKONEN

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Helsinki, April 1963.

R. P

INTRODUCTION

Patients with coronary heart disease frequently exhibit an abnormally high serum lipid content. This finding and the presence of an accelerated formation of atheroma in such endogenic defects of lipid metabolism as diabetes and essential hyperlipemia and hyper cholesterolemia support the view that coronary heart disease is in essence an error of lipid metabolism. In the course of research on atherosclerosis, the metabolism of each of various serum lipids and lipoproteins has in turn been thought to be altered the most in coronary heart disease.

Purely exogenous lipids, such as carotenoids (Thomson 1934, Blankenhorn *et al.* 1956) and tocopherol (Mc Cormick and McCluer 1960) have been found in human atheromas, thus suggesting that the atheromatic lipids are derived from circulating lipids and are not synthetized in the arterial wall According to the filtration theory of Page (1954) the primary process in atheroma formation is the lipid infilt ration, the fibrous tissue present in atheromas being a secondary reaction to the fat imbibition. Moreton (1947 1950) was the first to focus attention to the alimentary lipemia as a possible important factor in the deposition of lipid into the arterial wall.

In numerous investigations a prolonged and intensive postprandial lipemia has been found in patients with coronary heart disease This has been thought to be due to an impaired fat removal from circulation, reflecting an abnormal lipoprotein metabolism. The reported harmful effects of lipemia, i.e., the acceleration of blood coagula tion (Fullerton *et al.* 1953 O'Brien 1957) inhibition of fibrinolysis (Greig 1956) decrease of tissue oxygen ten sion (Joyner *et al.* 1960) and reduction of the coronary blood-flow (Regan *et al.* 1961) are also of great interest.

It was the object of the present investigation to study the behavior of purely exogenous lipids in the blood circulation of patients with coronary heart disease and of healthy persons. The fat-soluble vitamins A and E seemed to be well suited for this purpose, since they have been found to be present in human atheromas. Since these two vitamins are carried in a somewhat different manner by the serum lipoproteins (Krinsky *et al.* 1958, McCormick *et al.* 1960) it was considered probable that they would impart some information of the kinetics of lipoproteins.

REVIEW OF THE LITERATURE

SERUM CHOLESTEROL LEVEL IN CORONARY HEART DISEASE

The first reports of the presence of cholesterol in human atheroma are older than a hundred years. The great difference in the amount of cholesterol in atheromatic and intact aortas was observed much later (Windaus 1910) These basic observations as well as the production of atherosclerosis in rabbits by feeding a cholesterol-rich diet (Anitschkow 1913) focussed attention to cholesterol as an important factor in atherogenesis and led to a vast amount of research in this field.

Many surveys of this subject have been published. The following is only a brief summary of the outstanding studies and is far from complete

The observations concerning the simultaneous occurrence of hypercholesterolemia and clinical atherosclerosis increased with time, and in 1925 Mjassnikow showed that, of the various clinical manifestations of atherosclerosis, hypercholesterolemia particularly was associated with coronary heart disease. In a review published in 1935 it was stated that serum cholesterol increased with age and with the extent of atherosclerosis, and that its determination was of clinical value (Hurxthal and Hunt 1935)

The important role of a disturbed cholesterol metabolism in the development of atherosclerosis then began to be generally accepted and was supported by many clinical observations (Hirsch and Weinhouse 1943) The findings of Mjassnikow were confirmed by Davis *et al.* (1937) in a systematical clinical study The high incidence of coronary atherosclerosis in hypercholesterolemic xanthomatosis was stressed (Thannhauser and Magendantz 1938) It was emphasized that diabetic hyperlipidemia was often complicated by coronary heart disease (Rabinowitch 1935) Hypothyrotic patients frequently showed concomitant hypercholesterolemia and occlusive atheromatosis (Hurxthal and Hunt 1935) The hypercholesterolemia associated with coronary heart disease showed often a familial occurrence (Boas *et al.* 1948)

The first systematical clinical study by Davis *et al.*, cited above, revealed thus a significantly higher serum cholesterol content in patients with angina pectoris than in a somewhat younger control group. Similarly Morrison and co-workers (1948) found hypercholesterolemia in 68 per cent of 200 survi-

vors of myocardial infarction below the age of 60 years. The occurrence of hypercholesterolemia was less common (48 per cent) in the older group.

One of the best statistically analyzed studies is that of Gertler *et al.* (1950 a) Of 97 survivors of myocardial infarction, 24 had a cholesterol value below the mean control value and only 2 were below control mean — 1 S.D On the other hand 15.4 per cent of the matched controls exceeded the mean coronary cholesterol, while only 2 control persons exceeded the mean +1 S.D The significant difference observed was not age-dependent (Gertler *et al.* 1950 b) A similar significantly higher serum cholesterol mean content as well as a high phospholipid mean were found by Steiner et al. (1952) in 82 coronary patients, compared with 112 healthy individuals. The increase in serum lipid phosphorus was not proportional to the increase in serum cholesterol, and thus an elevation in the serum cholesterol/lipid phosphorus ratio became apparent. Supporting the view of Ahrens and Kunkel (1949) it was suggested that because of the shift in the ratio toward cholesterol, the amount of phospholipid may be insufficient to maintain all the cholesterol in solution. Further support to the significant role of a higher cholesterol mean level in coronary heart disease was brought by a statistically well analyzed study from England (Oliver and Boyd 1953) The 200 patients studied showed a significantly higher serum cholesterol level than the control persons through the entire age range from 30 to 70 years of age, with the exception of the sixth decade in women. The occurrence of true hypercholesterolemia was only found in a small coronary group below the age of 40 years. A significant elevation in the ratio of total cholesterol/phospholipid was seen also here and was regarded as an important gauge of a disturbance in the lipid metabolism in atherosclerosis. In a Swedish series published by Björck *et al.* (1957) young coronary patients (40—49 years) showed a significantly higher serum cholesterol level than healthy males from the same population. In higher age groups the difference appeared to be smaller or even absent when compared with the control group of Keys *et al.* (1950) It was suggested that the young coronary patients may have derived from a somewhat different population than the older ones.

By using an arbitrary cholesterol value of 241 mg/100 ml, more than 80 per cent of the men in the fourth decade in control and coronary groups were separated correctly (Little *et al.* 1956) Like the latter many other investigators have stressed the common occurrence of hypercholesterolemia in the younger coronary patients (Lawry *et al.* 1957 Björntorp and Malmcrona 1960 Scarborough et al. 1960 Albrink *et al.* 1961) On the other hand Carlson (1960 b) has postulated that the triglyceride metabolism was more disturbed in young coronary patients and that of cholesterol at a later age.

In the study of Lawry *et al.* (1957) the 75 per cent limit of healthy men of comparable age was used as discrimin

ator and 120 of 261 coronary patients exceeded this limit in serum chol esterol values. The serum cholesterol levels of 141 men with angina pectoris were not as high, while the values of female survivors were identical with those of male survivors. In contrast to the data from the Donner laboratory (Jones *et al.* 1951) Lawry and co-workers stressed that none of the lipid parameters studied (Sf 12—20 and 20—100 lipoproteins and cholesterol) showed any clear superiority in the prognostication of coronary heart disease. Mattingly *et al.* (1959) on the other hand, considered the serum chol esterol level more informative than the elaborate measurements of lipoproteins. In the study of Albrink *et al.* (1961) the cholesterol content of 260 mg/100 ml best separated 212 sur vivors of myocardial infarction from the healthy population. This was the concentration which in the Framingham study (Kannel *et al.* 1961) was the borderline for a marked increase of coronary heart disease. Only 5 per cent of young healthy men in the third decade exceeded this concentration. Half or more of the patients with coronary heart disease remained un identified, however The serum chol esterol level offered the best differen tiation between normal subjects and those with the disease under the age of 50 The serum cholesterol levels of 23 women who had had myocardial infarction were entirely comparable with those of men with this disease.

To evaluate the significance of the serum cholesterol content in coronary heart disease the National Advisory Heart Council organized a large-scale study in the United States in 1953 Four laboratories analyzed the chol esterol content of serum in 4,914 men. During a 2-year observation period, clinical manifestations of coronary heart disease developed in 82 men. The cholesterol measurements of all the laboratories combined showed a highly significant ability to place the subjects with definite events above the fiftieth percentile but not above the seventy fifth percentile Thus for the first time coronary heart disease was shown to be associated with an antecedent eleva tion of the serum cholesterol level (Gofman *et al.* 1956)

In 1948 the United States Public Health Service undertook a prospec tive study to investigate the incidence of coronary heart disease and factors related to its development. This study concerned 4,469 men 30 to 59 years of age in Framingham, Massachusetts. The serum cholesterol of all persons was determined. During a six year follow-up 186 men developed coronary heart disease, representing an over-all incidence of 36.3 per thousand. A sig nificantly higher mean cholesterol level was demonstrated among subjects who developed the disease than among the base population. The elevation was most marked in the men in the youngest age groups. It was concluded that a high cholesterol level increases the risk of development of coronary heart disease and that this risk in men 40 to 59 years of age is more than three-fold for individuals with serum cholesterol over 245 mg/100 ml than below 210 mg/100 ml (Kannel *et al.* 1961)

From the very beginning of research on cholesterol, the relationship between the serum cholesterol level and the extent of atherosclerosis found at autopsy has been a subject of investigation. The data available however are controversial. In an autopsy series of persons who had died of violence the serum cholesterol determined post mortem did not correlate with the aortic lipid content (Landé and Sperry 1936) In another study the cholesterol content of the aortic wall had a direct relationship to age but not to serum cholesterol (Faber 1946) The determination of cholesterol from post mortem blood samples was criticized by Morrison and Johnson (1950) They showed that the coronary arteries of patients who had died of acute coronary thrombosis contained, on an average four times as much cholesterol as those of control patients. Similarly hypercholesterolemia was found in most of the coronary patients, as compared to a normal average in the control group. The recent data of Paterson *et al.* (1960) again, showed that the severity of atherosclerosis was poorly correlated to the serum antemortem cholesterol level, except perhaps when it exceeded 300 mg/100 ml.

SERUM BASAL TRIGLYCERIDE LEVEL IN CORONARY HEART DISEASE

During the time that research of atherosclerosis has been carried on, different lipids or lipoprotein fractions have been thought to be the most atherogenic. The latest newcomer in the list of atherogenic lipids is neutral fat.

The alterations first observed in serum lipoproteins concerned the cholesterol-rich fraction Sf 10—20 (Gofman *et al.* 1950) Later however it appeared that the fraction Sf 12—400 of lower density showed a better correlation to clinical coronary heart disease (Gofman *et al.* 1954) suggesting an abnormally high serum triglyceride level (Havel and Carlson 1962) The presence of an abnormal metabolism of triglyceride was also supported by the observation of abnormalities in the alimentary hyperlipemia commonly seen in coronary patients, which will be described later In addition, attention has been drawn to a higher triglyceride content of the human coronary arteries as compared to the aorta (Böttcher *et al.* 1959)

The study of Hauss and Böhle (1955) stressed for the first time the common occurrence of hypertriglyceridemia in coronary heart disease. In 21 survivors of myocardial infarction, the serum triglyceride content was estimated by subtracting the other lipids from the total lipid value All patients exceeded the mean triglyceride level of age-matched controls, while the serum cholesterol values were in the upper limit of the control mean. The extensive studies of Schrade *et al.* (1959 1960 1961) supported this finding. Triglyceride was estimated by the sub-

traction method however similar data were obtained by absorption chromatography Their largest series (published in 1960) consisted of 452 atherosclerotic patients, 321 of whom had either angina pectoris or a healed myocardial infarction. Hyperlipidemia occurred in 80 per cent of coronary patients. The serum triglyceride content was 44.2 per cent higher in the whole series than in 60 healthy persons of comparable age. The values for total cholesterol, total lipids and phospholipids were 16.1, 29.2 and 17 4 per cent, respectively In a later publication (1961) they reported a 45 per cent increase of serum triglyceride with aging, calculated from two healthy male groups 18—42 and 46—71 years of age Nevertheless, the significant difference remained also when the coronary patient group was compared with the older control group. As a true control group Antonis and Bersohn (1960) used 57 healthy Bantu negroes with a low incidence of coronary heart disease. The mean serum triglyceride +1 S.D in the negroes was exceeded by 96 per cent of 23 European coronary patients in the same region, by 6 per cent of European male subjects below the age of 30 years, and by not less than 88 per cent of males over the age of 50 years. Since even the standard deviation increased with increasing age and serum triglyceride levels, the authors suggested the existence of two populations among older men, the one population consisting of the true healthy persons and the other of individuals with potent atherosclerosis. In a Swedish series studied by Carlson (1960 b) hypertriglyceridemia was characteristic of coronary patients below the age of 50 years, while the older patients showed hypercholesterolemia more often. Triglycerides were estimated by determining the glyceride glycerol. It was concluded that the coronary patients were composed of two different populations; the one with a primarily altered triglyceride metabolism and the other with a disturbed cholesterol metabolism.

Further light on this problem has been thrown by Albrink and co-workers (Albrink and Man 1959 Albrink *et al.* 1961, Albrink 1962) Triglycerides were calculated on the basis of the serum total esterified fatty acids. Contrary to the data of Carlson, hypertriglyceridemia was more common in patients over the age of 50 years (89 per cent) than in the younger patients, who showed a somewhat lower incidence (82 per cent) The younger patients showed a higher incidence of hypercholesterolemia, again in contrast to Carlson s data. The upper 95 per cent limit of healthy men of the age of 20—29 years was used as the normal value. This was exceeded, however by 40 per cent of healthy men over 40 years of age. To solve the problem of the common occurrence of hypertriglyceridemia among older healthy persons, Albrink and co-workers (1962) showed that hypertriglyceridemia was often associated with a weight gain of over 4.5 kg after the age of 25 years and/or with a positive family history of atherosclerosis.

Recently Berkowitz and Croll

(1962) supporting the earlier findings, stated that hypertriglyceridemia and impaired radioactive fat tolerance were the most characteristic abnormalities in lipid metabolism in coronary heart disease In 100 patients they observed hypercholesterolemia in 41 per cent and hypertriglyceridemia in 72 per cent, while 82 per cent exhibited an impaired fat tolerance

DISTRIBUTION OF CHOLESTEROL AND TRIGLYCERIDE IN SERUM LIPOPROTEINS IN CORONARY HEART DISEASE

In the basic observation of Gofman and co-workers (1950) attention was focussed on the elevated lipoproteins as the most significant abnormality in the blood of patients with coronary heart disease At first it was thought that the cholesterol rich lipoprotein Sf 10—20 was altered the most. Later however the Donner group broadened the lipoprotein analyses to comprise almost the entire beta lipoprotein spectrum Sf 0—12 12—400 (Jones *et al.* 1951 Gofman *et al.* 1952, 1954) In order to weight the atherogenicity of each fraction, an atherogenic index was developed. It was found to correlate to clinical coronary heart disease better than any individual lipoprotein fraction. The atherogenic power of Sf 12—400 has been weighted at 1.75 times that of the Sf 0—12 fraction (Gofman *et al.* 1954)

In the prospective study organized by the National Advisory Heart Council in the United States it appeared that there existed a prior elevation of Sf 20—100 but not of Sf 12—20 lipoprotein in the men who developed coronary heart disease during the 2-year period of observation (Gofman *et al.* 1956) The Donner and Eastern laboratories disagreed, however on the significance of these measurements, above all with respect to the predictive value of separating persons considered susceptible to coronary heart disease from the normal population. The existence of an elevated serum lipoprotein content in coronary patients has been confirmed by many other authors, but opinions differ concerning the superiority of the lipoprotein measurements as compared to the simple estimation of serum total cholesterol (Doyle *et al.* 1956, Little *et al.* 1956 Lawry *et al.* 1957 Mattingly *et al.* 1959 Page and Lewis 1959 Schlessinger *et al.* 1959)

Attempts have been made to clarify further the abnormal lipoprotein pattern in coronary heart disease by means of measurement of the lipid composition of the lipoproteins. Barr and co-workers (1951) were the first to show by lipoprotein lipid analyses abnormalities in also high density lipoproteins. They showed that in the sera of survivors of myocardial infarction the alpha-lipoproteins separated by the Cohn method showed a significant decrease in the cholesterol content,

while the beta-lipoprotein cholesterol was elevated. This finding was confirmed by Pratt (1952) in an prelimi nary study using the ultracentrifugal technique.

The method of eletrophoretical sepa ration for this purpose was introduced by Nikkilä (1952, 1953) The earlier observations were confirmed also in patients with a normal serum cholesterol content. If 70 per cent of the cholesterol bound to beta-lipoproteins was used as discriminator this level was exceeded by one-fourth of healthy individuals over 40 years of age. On the other hand, one-eighth of the coronary group was below this limit, thus showing a normal distribution pattern. Similar data have been obtained by Jencks *et al.* (1956) in paper electrophoresis and by Carlson (1960b) who isolated the lipoproteins in a glass powder column. In the latter study the measurement of the lipoprotein-lipid distribution, however separated the coronary patients from healthy population no better than did the serum cholesterol. Nor could Doyle and coworkers (1956) find any significant difference between healthy and coronary populations in the cholesterol or phospholipid content of the two major lipoproteins separated by Cohn fractionation. Serum cholesterol and phospholipids, however were significantly higher in the coronary group. It was concluded that in a biologically homogeneous population universally susceptible to coronary atherosclerosis, the blood lipoprotein pattern is neither quantitatively nor qualitatively a satisfactory index of atherosclerosis.

Confirming the earlier observations of an abnormal distribution of chol esterol and phospholipids in lipoproteins, Schettler *et al.* (1957) focussed attention on the high neutral fat concentration in the $alpha_1$ region of starch electrophoresis in the sera of coronary patients.

A similar tendency to a decrease of $alpha_1$ lipoprotein lipid with a concomitant increase of beta-lipoprotein lipid has been shown by the lipid staining technique in coronary patients (Antonini *et al.* 1953 Kroetz and Fischer 1954, Fasoli *et al.* 1957) The existence of a large pre-beta-lipid band, roughly equated with Sf 20-100 lipoprotein, was regarded by Smith (1957) as the most significant alteration in the sera of coronary patients. This method was shown to have an excellent property of separating the two groups.

Numerous data are thus available concerning alterations in the cholesterol and phospholipid levels in the two majo lipoprotein classes in the sera of coronary patients, and nearly all of them confirm one another Much work has been done with lipoprotein measurements by the ultracentrifugal technique However the distribution of different lipids in the beta-lipoprotein subfractions in coronary heart disease is but poorly known. In one study Havel and co-workers (1955) showed an elevation of the entire $D <$ 1.063 fraction in a few patients with atherosclerosis or with a disease predisposing to it. The increase in chol-

esterol and phospholipids was due to D 1.019—1.063, *to* D < 1.019 *or to* both fractions. Triglyceride estimations were not done The Donner group observed no significant alterations in the lipid composition of Sf 0—12, 20—400 and HDL 2—3 lipoproteins (Lindgren and Gofman 1957)

FAT LOADING TESTS IN THE STUDY OF LIPID METABOLISM IN CORONARY HEART DISEASE

Postprandial lipemia is caused by the newly absorbed neutral fat, which is in the form of fairly large particles often called chylomicra. The plasma lipid concentration curve obtained after ingestion of a lipid meal is a function of many simultaneously occurring biochemical processes fat absorption and transport in the plasma, the plasma lipid pool already present, and the rate of removal of the absorbed fat from plasma. The effect of the quality and quantity of the ingested fat is equally of importance Of all these processes, the mechanism of lipid transport and the disappearance of exogenic lipids from the plasma has been of particular interest in atherosclerosis research.

The first observations of the lactescence of the serum were made already in 18th century Much later however it was shown that the post prandial lactescence was due to small fat particles, termed chylomicra, which were newly absorbed neutral fat (Gage and Fish 1924 Frazer and Stewart 1937 Elkes *et al* 1939) At about the same time the first fat loading tests were performed in order to study the physiology of alimentary hyperlipemia (Nissen 1931 Page *et al* 1930 Man and Gildea 1932, Wechsler 1932) In 1934 Chaikoff and co-workers studied also the lipemic response in a case of disseminated cutaneous xanthomata.

The observation of Moreton (1947 1950) that newly absorbed fat in the postprandial plasma of healthy persons was in a state similar to that in the fasting plasma of hyperlipidemic patients led to a systematical study of postprandial hyperlipemia in atherosclerosis. Because atherosclerosis was common in such hyperlipidemic states as nephrosis, diabetes and essential xanthomatosis, Moreton stated that the cumulative effect of many fatty meals over a lifetime, by producing these transient showers of large lipid particles in the plasma, may be the underlying cause of intimal lipid deposition in human atherosclerosis.

After the observations of Moreton the study of postprandial hyperlipemia became one of the central problems in atherosclerosis research. It has been used to study the disturbed lipid metabolism often associated with coronary heart disease. Additionally attempts have been made to use the fat loading tests for discovering the latent defects susceptible to coronary heart disease which are not revealed by the analysis of fasting plasma.

Methods Used in the Study of Postprandial Lipemia

The method generally employed is to observe the plasma lipid concentration curve after the ingestion of a standardized fat meal. In recent years, par enteral administration has also come into use to eliminate intestinal absorption.

The large number of methods available for analyzing the lipemic plasma have been presented in detail in a recent review by Dole and Hamlin (1962) Only a few of them, however have been used widely in the quantitation of the lipemic response in fat loading tests.

In order to study the size of the lipid particles in postprandial plasma Moreton measured the scattering of light in a nephelometer The counting of chylomicra or measurement of their size under the microscope was used earlier by many authors (Becker *et al.* 1950 Zinn and Griffith 1950 Grüner and Hilden 1953 Schettler and Jobst 1955) Because of the many sources of error this method is no longer in use The determination of newly absorbed neut ral fat by measurement of the optical density in a photometer is one of the most common methods even today (Pomeranze and Beinfield 1951 White *et al.* 1951, Schwartz *et l.* 1952, Woldow *et al.* 1954, Barritt 1956, Eggstein and Schettler 1958, Mitchell and Bronte-Stewart 1959 Bouchier and Bronte-Stewart 1961 Brown *et al.* 1961) The direct chemical determination of triglyceride has been little used (Nikkilä and Konttinen 1962) but the estimation of plasma total fatty acids has been since earlier time in common use (Chaikoff *et al.* 1934 Hirsch and Carbonaro 1950 Pomeranze and Beinfield 1951, Eggstein and Schettler 1958, Brown 1961, Brown *et al.* 1961, Kingsbury *et al.* 1962)

The increase in the concentration of light lipoproteins (Sf 20-100) after an acute fat load has been known since the observations of Gofman *et al.* (1952) As a measure of lipoprotein concentration in fat loading tests, Wol dow and co-workers (1954) used the thymol turbidity test, and the changes in ultracentrifugal lipoprotein fractions during alimentary hyperlipemia were studied by Goldner *et al.* (1954) and Horlick (1957) The electrophoretical mobility of lipemic plasma was studied by Swahn (1953) Kunkel and Traut man (1956) and Jobst and Schettler (1956) The electrophoretical changes during the fat loading test were also studied in hyperlipidemic and atherosclerotic patients (Kuo *et al.* 1956) The lipid distribution in ultracentri fugal lipoprotein fractions after an acute fat load was investigated by Havel (1957) and the kinetics of differ ent lipoproteins after the administration of I^{131} labeled fat was the subject of an extensive study in various hyper lipidemic states by Kruger *et al.* (1960)

The postprandial lipemia depends on the nature of the ingested fat. Intensive lipemia followed the ingestion of fats containing long-chain fatty acids independent of the iodine value, while the lipemic response to medium-chain fatty acids was poor (Eggstein and Schettler 1958, 1959) Various oils

studied by Kingsbury et al. (1960) all caused triglyceridemia of at least 60 mg/100 ml, but there were differences in the response of the concentrations of serum cholesterol and phospholipids and in their ratio. Emulsification of the lipid in the study of Brown et al. (1961) gave an earlier peak in the plasma lipid concentration curve

The lipemia following the fat load depends also on the dietary habits preceding the test. The tissue distribution of the C[1] labeled chylomicra varied according to the nutritional status in laboratory animals used by Bragdon and Gordon (1958) The lipemic response to the fat load has been shown to diminish after restriction of the dietary fat (Pomeranze et al. 1954) and after corn oil supplements to the diet (Bronte-Stewart and Blackburn 1958) In one study however the alimentary hyperlipemia was reported to have been similar in three populations of different race and with quite different dietary habits (Bouchier and Bronte-Stewart 1961)

In addition to the natural butter fats, the test substances also in use are vitamin A, I^{131} labeled triolein and C -labeled tripalmitin. Cholesterol loading tests have also been performed (Wang 195.) but because of the poor lipemic response it seems not to be of great value in these tests.

Oral administration of I^{131}-labeled triolein was introduced by Thannhauser and Stanley (1949) and has been widely used since in the study of lipid metabolism in coronary heart disease (Likoff et al 1958 Hall et al 1959 Seller et al. 1959, Berkowitz 1960 Kruger et al. 1960 Metz et al. 1960 Berkowitz et al. 1961, Brown 1961 Brown et al. 1961, Edelman et al. 1961 George et al. 1961, Berkowitz and Croll 1962, Levine and Cohen 1962, Malamos et al. 1962) As pointed out by Thannhauser and Stanley the method has many advantages compared to the conventional fat loading. With this method it is possible to follow the disappearance of the labeled fat for a longer time. It was also thought that simultaneous determination of the specific activities of the free iodine and the protein-bound iodine gave some information on the lipid metabolism.

Although opinions differ concerning the similarity of the transport mechanism of neutral fat and vitamin A, as will be presented in the following section, vitamin A has been used as an indicator of neutral fat by some investigators in the study of lipid metabolism (Marit and Connor 1956, Beaumont et al. 1958, Beaumont and Beaumont 1960 a)

To eliminate the influence of the absorption component on the plasma concentration, and for better information on the plasma lipid disappearance rate, intravenous administration has been widely used since the experiments of Becker et al (1950) They administered lipemic plasma intravenously in a study of the influence of age on lipid disappearance from the plasma. The method used the most, however is the administration of I^{131} labeled triolein emulsion (Berkowitz et al. 1961, Feinberg et al. 1961, Balodimos et al. 1962, Mayfield et al. 1962) The clearance

rates of I^{131} labeled triolein and C^{14} labeled tripalmitin have been shown to be similar (Balodimos *et al.* 1962) Many investigators (Berkowitz *et al.* 1961, Bouchler and Bronte-Stewart 1961, Mashford and Nestel 1961) have also used an artificial oil emulsion (Lipomul)

Influence of Age on Postprandial Lipemia

Some controversy exists concerning the effect of age on postprandial lipemia. One of the earliest observations is that of Wechsler (1932) who stated that the curve of plasma total lipids was flat in young persons and ascending in persons of middle age and that a descending curve was obtained in association with arteriosclerosis. The difference in the chylomicron count seen in the two age groups after oral fat loading disappeared when lipemic plasma was given intravenously (Becker *et al.* 1950) The lipemia measured by determining the plasma total lipids lasted longer among older individuals in the study of Herzstein et al. (1953) Grüner and Hilden (1953) on the contrary observed a higher chylomicron count during postprandial lipemia in young individuals. However in studies by Barritt (1956) and Bouchler and Bronte-Stewart (1961) age did not at all influence the lipemic response A higher peak level of the plasma total fatty acids and of the chylomicron count in the older group than in the younger one was observed by Schettler and Jobst (1955) after fat loading. Similarly a faster plasma clearance rate of lipids in the younger group as compared with the older one was found in the study of Brown *et al.* (1961) The disappearance rate of intravenously administered I^{131} labeled triolein correlated intimately with age in the study of Mayfield *et al.* (1962) The disappearance rate became slower with the increase of age independently of the clinical manifestations of atherosclerosis. They concluded that the slower lipid clearance rate observed by many authors in atherosclerotic patients reflects the age difference rather than a metabolic defect.

Meager data are available on the influence of age on alimentary lipemia in coronary heart disease. The expected abnormality in the I^{131} curve in diabetic and coronary patients over the age of 60 years was not found by Sandberg *et al.* (1960) and Edelman *et al.* (1961) In the study of Mayfield *et al.* (1962) however the age correlation in coronary patients continued throughout the entire age span up to the age of 70 years, as was stated above. On the other hand, Barritt (1956) and Seller et al. (1959) observed among coronary patients no correlation between age and lipemia.

Effect of Physical Activity on Postprandial Lipemia

Methodologically the effect of physical activity is of some importance, since in many experiments the control group consists of subjects doing their normal daily work, while the patient

protein pattern. On the other hand, only two subjects in the control group responded abnormally The ratio of peak protein-bound activity to peak supernatant activity was also significantly higher in patients who had an abnormal radioactivity curve. In another study the I^{131}-triolein clearance time (time required for the serum radioactivity to fall to half its peak value) was estimated in 40 patients with coronary heart disease (Hall *et al.* 1959) When this value was compared with the serum cholesterol content it appeared that both parameters were abnormal in 25 cases, the clearance rate in 10 cases only and the serum cholesterol in 4 cases only

However no significant difference was obtained in the plasma radioactivity curves after the administration of I^{131}-labeled triolein to coronary and age-matched control groups (Metz *et al.* 1960) They studied also the behavior of labeled fat in a group of Bantu negroes representing a true control group having a rare occurrence of coronary heart disease. The result was unexpected because the plasma activity in coronary patients was significantly higher at 6 8 and 12 hours, whereas the Bantus showed a higher plasma activity in 36 hours. They suggested that this was due to a liver dysfunction common among negroes or to an earlier feed-back phenomenon resulting from a faster clearance rate of the negroes.

Brown *et al.* (1961) examined the postprandial lipemia of 31 survivors of myocardial infarction and two control groups (age-matched subjects and young students) by determining the optical density the total fatty acids and the I^{131}-activity pattern. There was a close relationship between the three measurements however the peak level of radioactivity appeared a little later than the peak level of optical density or of total fatty acids. It appeared that the measurement of lipid bound radioactivity and the absolute increase of serum total fatty acids 9 hours after the test dose gave the best separation between the coronary group and the age-matched control group. The separation was more effective than by analysis of the fasting plasma lipids; 13 per cent of coronary patients showed, however a normal response. In a recent study of Berkowitz and Croll (1962) the I^{131}-tolerance was compared with the fasting lipid content of plasma in 100 patients with coronary heart disease. The incidence of hypercholesterolemia was 41 per cent, 72 per cent showed hypertriglyceridemia, while 82 had an abnormal I^{131} tolerance. An excellent correlation existed between the fasting triglyceride level and the radioactivity curve. In recent studies of small series, the abnormality to clear labeled fat in coronary heart disease has been confirmed by two investigator teams (Levine and Cohen 1962, Malamos *et al.* 1962)

An analysis of the kinetics of I^{131} labeled fat in coronary heart disease has been presented by George *et al.* (1961) They suggested that the disappearance of ingested labeled fat was composed of a rapid phase during which 95 per cent of the ingested fat is

cleared and of a slower component accounting for 5 per cent representing either retained or recirculating fat. The abnormality in the fat removal in coronary heart disease was present in the latter phase.

Disappearance Rate of Intravenously Administered Fat in Coronary Heart Disease

Contrary to the oral fat loading test, fat emulsions given intravenously appear to clear with an equal efficacy in patients with coronary heart disease and in healthy persons. The oral administration of an artificial fat emulsion (Lipomul) induced in coronary patients a more prolonged and intensive lipemia than in controls when measured by the plasma optical density (Bouchier and Bronte-Stewart 1961) When the fat emulsion was given intravenously the lipid disappearance rate was identical in the two groups. Similar results were obtained by Mashford and Nestel (1961) The clearance rate of intravenously administered I^{131}-triolein emulsion was also the same in patients with coronary heart disease and healthy individuals (Feinberg *et al.* 1961) When patients with an abnormal oral I^{131}-fat curve were tested by the intravenous method, it appeared that abnormality was shown by 25 per cent only The results were the same with orally and intravenously administered fat emulsions (Lipomul) (Berkowitz *et al.* 1961) The plasma protein-bound radioactivity curves obtained after intravenous administration of I^{131}-triolein and C -tripalmitin were similar in patients with coronary heart disease patients with diabetes and healthy persons (Balodimos *et al.* 1962) This was confirmed in a recent rapport of Mayfield *et al.* (1962)

Fat Tolerance Studies in Hyperlipidemic States

A large amount of data are available on alimentary lipemia in hyperlipidemic states. The results of studies of diabetic patients vary considerably probably according to the serum lipid values, diabetic complications and severity of the disease (Hirsch and Carbonaro 1950 Camelin *et al.* 1954, Beaumont *et al.* 1958, Beaumont and Beaumont 1960 a, Sandberg *et al.* 1960 Balodimos *et al.* 1962) The similarity in coronary and diabetic patients of the results of tests with I^{131} labeled fat has been emphasized by Sandberg *et al.* (1960)

In spite of some controversy most authors agree that the alimentary lipemia tends to be normal in pure hypercholesterolemia (Chaikoff *et al.* 1934, Thannhauser and Stanley 1949 Kuo *et al.* 1956, Beaumont *et al.* 1958, Likoff *et al* 1958, Kruger *et al.* 1960) In an excellent study of lipoprotein kinetics in various hyperlipidemis states, Kruger and co-workers, using labeled triolein, showed that in all the subjects the labeled lipid appeared almost exclusively in the chylomicra and the Sf 10—400 lipoprotein fraction. They showed also that in hypercholesterolemic patients with an elevated Sf 3—9 lipoprotein fraction the labeled lipid peaked

early and was essentially cleared from the blood within 24 hours, as in control cases. Patients with hyperlipemia char acterized by an elevated Sf 10—400 lipoprotein fraction exhibited a delayed peaking of the labeled lipid, which was still considerably elevated after 24 hours. A similar gross abnormality in the lipid curves of patients with hyper lipemia has been observed by many other authors (Thannhauser and Stanley 1949 Martt and Connor 1956 Beaumont *et al.* 1958, Hall *et al.* 1959 Beaumont and Beaumont 1960 a, Brown 1961 Meng 1961, Sigler and Rubini 1961)

PLASMA VITAMIN A AND ITS RELATION TO VITAMIN A METABOLISM

Vitamin A has been used since the 1930 s as an indicator in the study of fat absorption owing to its fat solubility and relative ease of determination (Chesney and McCoord 1934) The vitamin A test is probably the most widely used test in the study of malabsorption. However it has rarely been used in the study of fat metabolism in cases of hyperlipidemia.

Intestinal Absorption of Vitamin A

The absorption of vitamin A across the human intestine is an active process that requires energy The energy is produced by oxidative phosphorylation (Loran *et al.* 1961)

In nature, vitamin A occurs in an esterified form. The first stage of vitamin A absorption appears to be a fairly complete hydrolysis in the in testinal lumen or on the surface of the epithelial cell (Loran *et al.* 1961 Mahadevan and Ganguly 1961)

It has been shown in animal experi ments that at the stage of absorption the mesenterial lymph fluid and the intestinal wall contain, in addition to vitamin A in the form of free alcohol, vitamin A esterified only with long chain fatty acids (Mahadevan *et al.* 1959) Re-esterification of vitamin A with palmitic acid has also been observed in the human intestine in *in vitro* experiments (Loran *et al* 1961) According to the generally accepted opinion today the transport of vitamin A from the intestine onward occurs esterified with long-chain fatty acids.

The absorption through the thoracic duct is well established. Drummond and his co-workers (1935) demonstrated in a patient with chylothorax that vitamin A is quite completely absorbed through the lymphatic system. They also observed that vitamin A administered as free alcohol was found in the esterified form in the chyle. A par allel rise in the vitamin A concentrations of serum and chyle after a dose of 500 000 units of vitamin A has been observed in another patient with chylothorax (Beaumont and Beaumont 1960 b) The absorption of vitamin A

through the thoracic duct has also been demonstrated in experimental animals (Eden and Sellers 1949) They also observed that the vitamin A content of portal and systemic blood was equal and that after the administration of vitamin A the increase in the portal blood was slightly lower

After a single dose of vitamin A the concentration in blood rises, depending chiefly on the ester fraction regardless of the form in which the vitamin is administered. This has been shown by many investigators in human subjects (Hoch 1946 Popper *et al.* 1948 Week and Sevigne 1950 Dost and Rind 1957 Krinsky *et al.* 1958) and in laboratory animals (Ganguly and Krinsky 1953) A flat increase of the alcohol fraction has also been observed by some investigators (Hoch and Hoch 1946, Ganguly and Krinsky 1953)

The peak in the serum concentration after a single dose of vitamin A is reached in about 3 to 6 hours, and the level of the peak depends on the dosage. However Dost and Rind (1957) reported that the height of the peak is not determined by the size of the dose alone, the vitamin disappearance rate being equally important. Free alcohol causes a more intensive and more rapid rise than the ester (Week and Sevigne 1950)

Vitamin A is absorbed much better in aqueous dispersion than in oily medium (Barnes *et al.* 1950 Sobel 1952, Moore 1957) In rats most of the absorption takes place in the upper jejunum with aqueous medium and in the lower jejunum with ily medium (Sobel 1952) Evidently bile is necessary in the absorption of vitamin A (Moore 1957)

Plasma Vitamin A Level

Despite the fact, presented above that a single dose of vitamin A is followed by a rise only in the ester fraction of the serum, c. 80—90 per cent of vitamin A in postabsorptive plasma is free alcohol (Hoch and Hoch 1946 Popper *et al.* 1948, Week and Sevigne 1950 Ganguly and Krinsky 1953 Dost and Rind 1957)

The regulation of the plasma vitamin A alcohol content is poorly understood. It is quite evident that vitamin A is absorbed esterified and transported to the liver which contains about 90 per cent of the body stores. The circulating free alcohol is independent of the liver stores, howeve (Glover *et al.* 1947 Ganguly and Krinsky 1953 High and Wilson 1956) Liver is not capable of hydrolyzing the long-chain fatty acid esters of vitamin A, and this probably occurs in the extrahepatic tissues (Ganguly 1960)

Much data are available in the literature on the plasma content of vitamin A in healthy individuals. There are considerable variations in the different reports, however In his monograph, Moore (1957) calculated the average of reported concentrations, which in 1,040 subjects was 131 I.U per 100 ml of plasma, being within the limits of 91—201 I.U per 100 ml plasma. The averages in some of the largest materials were as follows:

Investigator	Number of Subjects	Mean of Plasma Vitamin A I.U./100 ml.	Range
Leitner et al. (1960 a)			
women	526	142	54—259 (95 %)
men	742	174	70—305 (95 %)
Vetter (1958)	220	226	175—275
Saksela (1940)	214	191	105—315
Abels *et al.* (1941)	124	160	
Pitkänen (1944)			
women	72	201	87—374

There is good agreement concerning the influence of sex on the plasma vitamin A content. The level of plasma vitamin A in male subjects usually exceeds that for the female by a significant amount (Kimble 1939 Abels *et al.* 1941, Week and Sevigne 1950 Leitner *et al.* 1960 a)

Data on the influence of age on the vitamin A content, on the other hand are conflicting One of the early studies of the subject indicated a considerable decline with age (Schneider and Widmann 1935) No clear correlation to age was seen in the series of Saksela (1940) consisting of subjects 12—50 years of age. Vetter (1958) reached the same conclusion. However in the largest published series (Leitner *et al.* 1960 a) the mean plasma vitamin A levels increased with age up to the sixth decade in males and the seventh decade in females, at which time both sexes reached the same level.

The vitamin A levels are higher during the summer and spring seasons than in the late fall (Saksela 1940 Vetter 1958, Leitner *et al.* 1960 a) whereas no 24-hour fluctuations have been found (Lindqvist 1938 Kimble 1939)

Transport of Vitamin A in the Blood

The importance of a preliminary saponification of the plasma, apparently to remove vitamin A from some complex, was shown by Lindqvist (1938) A globulin complex was suggested by Pett and LePage (1940) because of the property of plasma vitamin A to precipitate with alcohol. After freezing of the plasma at —25 C, the amount extractable with ether rose significantly in the study of Dzialoszynski *et al.* (1945) They thought that the binding protein was albumin. In 1950 Oncley and co-workers found that beta-carotene, the provitamin A, was carried in plasma by beta-lipoproteins. In ultracentrifugal studies Hack (1956) observed, however that vitamin A and carotenoids were concentrated in different protein layers.

A number of experiments with man and animals have brought out that the vitamin A esters and alcohol are carried by different proteins (Ganguly *et al.* 1952, Garbers 1958 Krinsky *et al.* 1958, Krishnamurthy *et al.* 1958, Garbers *et al.* 1960)

Krinsky *et al.* (1958) in their studies of the transport of vitamin A in plasma after a single dose of the vitamin ob-

served no rise in the alcohol fraction. According to the results of ultracentrifugation, most of the free alcohol was in fraction D > 1.063 and a small amount in fraction Sf 3—9; the latter finding, however, they considered to be due to contamination. Immunologic experiments and ethanol fractionation suggested that the protein binding the vitamin A alcohol was not albumin. In experiments with rats, using C[14] labeled vitamin A, Garbers and co-workers (Garbers 1958 and Garbers *et al.* 1960) showed that vitamin A alcohol was bound to alpha$_1$-globulin and not to lipoproteins.

Evidently the esterified vitamin A is carried in plasma by lipoproteins, chiefly by low density lipoproteins. In a careful study, Krinsky *et al.* (1958) showed that after a single dose of vitamin A the absorbed vitamin ester was carried primarily in the Sf 10—100 lipoproteins. The chylomicra contained only a small part of the absorbed esters, suggesting a transport mechanism differing from that of neutral fat. There was also a small amount of vitamin A esters in the Sf 3—9 lipoprotein fraction.

The French investigators (Beaumont and Beaumont 1960 a) criticized this transport mechanism, however. They showed that vitamin A esters were primarily carried by chylomicra and that the newly absorbed vitamin appeared in beta-lipoproteins 6 hours after the ingestion of vitamin.

Very interesting is the observation by Schrieck and Kunkel (1956) of the influence of heparin on the different vitamin A fractions after vitamin A loading. There was a considerable fall in the total plasma vitamin A, with a rise in the free alcohol and a marked decrease in the ester fraction. Electrophoretic and ultracentrifugal analyses of the plasma indicated that heparin caused a partial shift of esterified vitamin from low density alpha$_2$-lipoproteins to beta-lipoproteins. Free vitamin alcohol appeared in the alpha$_1$-albumin fraction.

Storage of Vitamin A in the Liver

The vitamin A is distributed throughout the body. However, the liver contains 90 per cent of the body stores, the average concentration being about 250 I.U. per gm of liver tissue (Moore 1957). The liver is able to store a considerable amount of the vitamin esters but only a small portion of free alcohol (Ganguly 1960). In laboratory animals the liver vitamin A was esterified with palmitate only, regardless of the form in which the vitamin was fed (Mahadevan and Ganguly 1961). They thought that this was due to a selective binding property of the liproproteins. After the feeding of vitamin A, the liver of laboratory animals continued to store the ester fraction for a long time, but the alcohol fraction reached its maximum in 3 to 5 hours and did not increase thereafter (Ganguly and Krinsky 1953). It has been suggested that the esters are phagocytized directly by the Kupffer cells from the circulation. The free alcohol first underwent hydrolysis in the plasma and was then stored in parenchymal cells (Glover and Morton 1948). To confirm this, Krishna-

murthy and Ganguly (1956) showed that the blockage of the reticulo-endothelial system in laboratory animals led to a decrease in the liver ester fraction after vitamin A feeding. The blocking did not influence the liver alcohol fraction, however. In another study the blockage led to a marked slowing down of the plasma disappearance rate of vitamin A (Brown *et al.* 1952).

Metabolism of Vitamin A in Hyperlipidemic States

The exact role of vitamin A in lipid metabolism has not yet been established. The study of Lindqvist (1938) showed a significant parallelism in the serum cholesterol and vitamin A levels in response to iodine treatment in hyperthyroid patients. He suggested also that the simultaneous occurrence of hypercholesterolemia and hypervitaminosis A in hypothyrosis had a common cause, probably in a pathologic affinity to serum. In the reports of Wendt (1935) and Josephs (1942) a transient elevation of serum total cholesterol after vitamin A feeding was found in laboratory animals and human subjects. Similarly during treatment with vitamin A and vitamin E of 3 weeks' duration Vannotti and Gervasoni (1957) observed in atherosclerotic patients and healthy subjects an increase of plasma total lipids and cholesterol. In another experiment, vitamin A showed an antisclerotic effect on aortic atheromas in hens; no change, however, was observed in the serum cholesterol levels (Weitzel 1957). Contrary to earlier results Kinley and Krause (1959) reported that treatment with 100 000 units of vitamin A daily resulted in a decrease of 20 to 175 mg/100 ml of the serum cholesterol in hypercholesterolemic survivors of myocardial infarction. Vitamin A had no influence on the normocholesterolemic subjects.

The importance of the function of the reticulo-endothelial system has been pointed out by Brown and coworkers (1952) as the common link in the metabolism of cholesterol and vitamin A. They observed an elevation of serum cholesterol and vitamin A levels in guinea-pigs after blockage of the reticulo-endothelial system with Thorotrast.

The presence of hypercarotenemia in various hyperlipidemias has been observed by many investigators (Rabinowitz *et al.* 1930, Wendt 1935, Ralli *et al.* 1936, Mandelbaum *et al.* 1942, Kimble *et al.* 1946, Cohen 1958, Blankenhorn 1960). The mechanism of hypercarotenemia is in many instances obscure. It has been suggested that the conversion of beta-carotene to vitamin A is impaired in diabetes and hypothyroidism (Ralli *et al.* 1936, Cohen 1958). In a large study of 116 diabetic patients (Kimble *et al.* 1946) the simultaneous occurrence of high blood carotene and low vitamin A levels was, however, unusual.

It also has long been known, that carotenoid pigments are responsible for the yellow color of early atheromas (Thomson 1934). Very interesting was the observation of Blankenhorn *et al.*

(1956) that human atherosclerotic lesions contained carotenoids in direct proportion to the severity of the atherosclerosis. He could also demonstrate that carotene feeding augmented the carotene content of xanthomas in a patient (Blankenhorn 1960)

Hypervitaminosis A has been a common finding in many cases of nephrosis (Saksela 1940 Popper *et al.* 1948 Kagan *et al.* 1950 Cohen 1958) The elevation depends on the ester fraction (Popper *et al.* 1948) Popper *et al* suggested also that the elevation was due to increased solubility in the serum. The vitamin A metabolism in the nephrotic syndrome of children has been studied by Kagan *et al.* (1950) who observed the highest peak level in these children after the administration of vitamin A. The slow plasma disappearance rate also observed was thought to be due to failure by the body to store the plasma vitamin. They *noted also an increase in the plasma* total lipid level after the ingestion of vitamin A and connected this lipid mobilization to the vitamin retention in the plasma. The hypervitaminosis A in hyperlipidemic states was explained by Krinsky *et al.* (1958) as a block within the reticulo-endothelial system in the normal metabolism of the Sf 10—100 lipoproteins.

There is some controversy concerning the occurrence of hypervitaminosis A in hypothyroid states. As was stated above it has been suggested to be a defect in the conversion of carotene to vitamin A (Cohen 1958) According to his study the vitamin A blood levels were chiefly within normal limits. High levels of vitamin A, however have been reported by many investigators (Wendt 1935 Lindqvist 1938, Saksela 1940) A slow clearance rate after vitamin A loading was seen by Beaumont *et al.* (1958)

Among diabetic patients the plasma vitamin A values reported in the literature vary from low to high levels (Wendt 1935 Lindqvist 1938, Saksela 1940 Murril *et al.* 1941, Kimble *et al.* 1946, Beaumont *et al.* 1958, Cohen 1958) In the study of Wendt (1935) hypervitaminosis in diabetics was often accompanied by hypercholesterolemia. Insulin treatment did not alter the vitamin level. No correlation, however between the serum total lipids and the carotene or vitamin A level occurred in the study of Kimble *et al.* (1946)

An abnormally high plasma concentration curve and delayed disappearance of vitamin A was observed by Martt and Connor (1956) in a case *of idiopathic hyperlipemia* associated with coronary atherosclerosis. The vitamin A metabolism in coronary heart disease with or without high serum lipid levels has been studied extensively by the French investigators (Beaumont *et al.* 1958, Beaumont and Ardaillou 1959 Beaumont and Lenègre 1959 Beaumont and Beaumont 1960 a, 1961) There were 23 abnormally high responses to vitamin A loading in 54 patients with angina pectoris. Of these 1 patient had myxedema, 8 essential hyperlipemia, and in 4 patients the lipid pattern was normal, while the others had moderately increased lipid values. A striking observation was that all the patients with

primary hypercholesterolemia responded normally The high concentration of vitamin A in the chylomicron fraction was characteristic of the hyperlipemia group. There was still a marked retention of this fraction 24 hours after the loading. The investigators concluded that the common occurrence of high vitamin A concentration curves reflects a faulty chylomicron metabolism in coronary heart disease.

METABOLISM OF ALPHA TOCOPHEROL

Alpha-tocopherol, which comprises most of the tocopherol pool of the human body (Quaife *et al.* 1949 Dju *et al.* 1958) is a natural vitamin E. Although vitamin E is regarded as a typical vitamin, the deficiency of which produces characteristic deficiency symptoms in experimental animals, its function is not known. Some investigators consider tocopherol to be a non-specific anti-oxidant, since it is a readily oxidizing substance and since some of the deficiency symptoms disappear after the administration of synthetic anti-oxidants. On the other hand, a large group of investigators support the opinion that tocopherol plays a fully specific part in certain biochemical reactions, as do other vitamins (Schwartz 1961) In human pathophysiology the significance of vitamin E is slight. In induced vitamin E deficiency in humans, however an increased peroxide hemolysis has been observed (Horwitt 1960)

Intestinal Absorption of Tocopherol

After a single dose of tocopherol the serum tocopherol concentration rises slowly and reaches a peak value at 6 to 12 hours, according to different authors (Quaife and Harris 1944, Popper *et al.* 1949 Klatskin and Krehl 1950 Week *et al.* 1952, Pomeranze and Lucarello 1953, Beckman 1955 McCormick *et al.* 1960)

According to the studies of Week and his co-wokers (1952) the shape of the serum concentration curve depends upon the form in which the tocopherol is administered. Free tocopherol produces a more rapid rise and a higher peak than the ester form, but in both cases the increase in concentration is due to free tocopherol. Pomeranze and Lucarello (1953) observed that the simultaneous ingestion of fat increases the absorption of tocopherol, and that a fat deficit diet preceding tocopherol loading decreases absorption. McCormick *et al.* (1960) obtained a two-peaked concentration curve if the test subject had a meal during the loading test.

Attention has been paid in several connections to the similarity of tocopherol and fat absorptions. Darby and co-workers (1946) studied the absorption of tocopherol in patients with sprue and observed that both the fasting value and the absorption curve

were low Low tocopherol values have also been seen in other conditions of deficient absorption (Darby *et al.* 1949) Popper and his group (1949) reported poor tocopherol absorption in acute hepatitis and hepatic cirrhosis. In detailed studies of tocopherol absorption in liver disease Klatskin and co-workers (1950 1952 a, 1952 b) obtained a lower curve in hepatic cirrhosis than in control persons, but the surface area of the concentration curve was of the same size The tocopherol concentration in the feces of cirrhotic patients was lower however and the administration of tocopherol in aqueous dispersion did not improve the absorption. They therefore suggested that the low plasma tocopherol values seen in cirrhotic patients were not a result of poo absorption.

The presence of tocopherol in feces has been observed in absorption studies (Cuthbertson *et al.* 1940 Hines and Mattil 1943 Hickman *et al.* 1944, Harris 1950 Klatskin and Molander 1952 b Rosenkranz *et al.* 1951, 1953) In laboratory animals a part of parenterally given C labeled tocopherol succinate was excreted in feces (Simon *et al.* 1956 a) This confirmed the earlier observations that there is an enterohepatic circulation of tocopherol, as in the case of cholesterol. Popper and his group (1949) had observed earlier that in patients with a biliary fistula the tocopherol concentration of the bile was of the same order of magnitude as that of the serum. A similar observation was reported by Klatskin and Molander (1952 a) in patients with hepatic cirrhosis, who stated, however that the administration of tocopherol did not raise the bile tocopherol level.

Plasma Tocopherol Level

Since the early 1940 s, much data has been published on the tocopherol content of the serum. Alpha-tocopherol makes up about 80 to 90 per cent of the total plasma tocopherol content (Quaife *et al.* 1949) and about one-third is in quinone form (Scudi and Buhs 1942, McCormick *et al.* 1960) The greater proportion of plasma tocopherol is free alcohol and only about 10 per cent is esterified (Rindi and Perri 1957) Furthermore, the tocopherol is entirely bound to the lipoproteins (Lewis *et al.* 1954 McCormick *et al.* 1960)

Many surveys are available of the plasma tocopherol level (Rauramo 1946, Beckman 1955, Feldheim 1957 Harris *et al.* 1961) The largest series that have been published are the following

	Number of Subjects	*Mean of Serum Alpha-Tocopherol mg/L*	sd
Leitner et al. (1960 b)	[illegible]	10.5	±2.3
Harris et al. (1961)	197	10.5	±3.2
Chieffi and Kirk (1951)	183	9.8	±3.0
Engel (1949)	122	7.7	±3.5
Kramer (1955) women	116	9.9	±2.5

3

The mean serum tocopherol value of all the cases published in the literature is 10.1 ± 2.4 mg/L, according to Harris *et al.* (1961) with a range from 0.8 to 20 mg/L, according to Feldheim (1957) showing thus a considerable variation.

According to the large survey of the literature prepared by Beckman (1955) the serum tocopherol content is lower in children than in adults. A positive age trend is also present in adults (Darby *et al.* 1949 Lemley *et al.* 1949 Chieffi and Kirk 1951, Leitner *et al.* 1960 b)

In the study of Rauramo (1946) women had a significantly higher serum tocopherol content than men, as also was the case in the study of Chieffi and Kirk (1951) No difference in this respect was present in Leitner's series when calculated from his total series. However the different age groups showed significant differences in both directions.

Transport of Tocopherol in the Blood

Very little is known concerning the fate of tocopherol after its absorption from the intestine into the blood circulation. Sternberg and Pascoe-Dawson (1959) in studying the metabolism of tocopherol in experimental animals with C^{1}-labeled alpha-tocopherol succinate found an equal radioactivity in the different lipoproteins in both the portal venous blood and the aortic blood. The absorption would thus take place by way of the thoracic duct.

Tocopherol evidently is completely bound to the lipoproteins (Lewis *et al.* 1954 Sternberg and Pascoe-Dawson 1959 McCormick *et al.* 1960) On the other hand, Ames and Risley (1949) were able to produce a tocopherol protein complex in *in vitro* experiments, and Voth and Miller (1958) demonstrated the existence of a fairly strong affinity between tocopherol and bovine albumin. On the basis of these studies it was suggested that tocopherol is bound to all proteins also under *in vivo* conditions. Evidence speaking against this opinion, however are the studies of Sternberg and Pascoe-Dawson (1959) using labeled tocopherol, in which no radioactivity was found in the albumin fraction.

Data on the binding of tocopherol to the various lipoprotein fractions are contradictory According to Lewis *et al.*, tocopherol was bound under fasting conditions mainly to the high density lipoproteins (c. 54 per cent) while the beta-lipoproteins contained only 20 per cent of the plasma tocopherol. In the study of McCormick *et al.*, however most of the plasma tocopherol was in the Sf 3—9 fraction. After the ingestion of tocopherol the serum concentration curve was two-peaked, in similarity to the curve for the chylomicron and Sf 10—400 fractions. The first peak was reached about 3—4 hours and the second peak about 12 hours after the intake of tocopherol. The tocopherol contents of fractions Sf 3—9 and of the high density lipoproteins increased at a slow rate and reached maximum in about 8—10 hours. On the basis of these results the investigators concluded that te tocopherol was converted from the lighter lipo-

proteins into Sf 3—9 and high density lipoproteins.

In experiments with rats, using labeled tocopherol, the radioactivity was distributed in paper electrophoresis as follows: Chylomicron fraction, 27.5 per cent, $alpha_1$ and beta-fractions, 58.4 per cent, and the remainder in the $alpha_2$-fraction (Sternberg and Pascoe-Dawson 1959)

The plasma disappearance rate of tocopherol is slow According to Beckman (1955) the plasma tocopherol content at 24 hours after tocopherol loading was higher than the fasting value if the dose exceeded 200 mg. In rats the plasma half-life of C[1] labeled alpha-tocopherol was 60 hours (Sternberg and Pascoe-Dawson 1959)

Tissue Storage of Tocopherol

Most of the tocopherol is stored in tissues in the alpha form, although the latter comprises only one-half of the total vitamin E intake (Quaife *et al.* 1949 Dju *et al.* 1958) Accordingly alpha-tocopherol has been found to be absorbed from the intestine better than the next most common gamma-tocopherol (Quaife *et al.* 1949) In the opinion of Dju, tocopherol is incorporated into the cells as a natural part of the exogenous fat in connection with the normal lipid metabolism of the cells.

Tocopherol is distributed throughout the body the largest deposits being in adipose tissue. However the highest concentrations per gram of fat are found in the plasma, hypophysis, adrenals and gonads (2.0 1.2, 1.0 and 0 7 mg, respectively of tocopherol per gram of fat) Considerably lower concentrations are found in the liver heart and skeletal musculature in which it is about 0.3 mg per gram of fat. The tocopherol content of the tissues increases with age and attains the maximum at the age of 20—30 years, after which it gradually declines until in old age it reverts to the childhood level (Dju *et al.* 1958)

Chemical Transformations of Alpha-Tocopherol in the Metabolic Process

Tocopherol is evidently excreted in the bile into the intestine. It probably is not excreted unchanged in the urine even if a reducing substance has been encountered in the urine after large doses of tocopherol and has been interpreted to be tocopherol or its quinone (Cuthbertson *et al.*, 1940 Rosenkranz *et al.*, 1953) According to Simon *et al.* (1956 a) and Sternberg and Pascoe-Dawson (1959) however tocopherol metabolites in the form of glucuronides are probably excreted in the urine.

It is the prevailing opinion that the first stage of tocopherol metabolism is the oxidation to tocopherol quinone, followed by reduction to hydroquinone (Scudi and Buhs 1942, Rosenkranz *et al.* 1953, Simon *et al.* 1956 a, Diplock *et al.* 1960 McCormick *et al.* 1960) Complete unanimity has not been reached on this point, however since these metabolites have not been encountered by some investigators in the tissues of

experimental animals (Hines and Mattil 1943, Pollard and Bieri 1959 Alaupović *et al.* 1961)

Simon and his co-workers (1956 b) isolated from the urine two metabolites and identified them as 2 (3-hydroxy-3-methyl-5-carboxypentyl)-3 5 6, trimethyl benzoquinone and its gamma lactone They also advanced the theory that tocopherol is first oxidized into quinone, reduced to hydroquinone and conjugated with glucuronic acid The last methyl group in the side chain is then oxidized to a carboxyl group and conjugated with CoeA. By means of beta-oxidation the side chain is shortened to contain six carbon atoms, after which lactonization and excretion in the urine occur Alaupović *et al.* (1961) were unable to isolate these metabolites from the liver of experimental animals, but they presented instead three new metabolites, the chemical structure of which is still unclear From the mitochondria of the rabbit liver Martius and Costelli (1957) isolated, in addition to unchanged alpha-tocopherol, a metabolite which they regarded as the active form of tocopherol. the trimethyl phythyl benzoquinone

***Relation of Tocopherol to Cholesterol* and Other Lipids**

Numerous animal experiments have revealed a rise in the muscle and serum cholesterol concentrations in vitamin E deficiency Oppenheimer *et al.* 1958) further observed a decrease in the plasma alpha-lipoprotein cholesterol but an increase in the beta lipoprotein cholesterol, giving the net result of an increased total cholesterol level in the plasma. Shull *et al.* (1958) and Alfin-Slater (1960) again, were able to inhibit with synthetic antioxidants the development of muscular dystrophy in vitamin E deficiency but obtained no effect on the cholesterol metabolism. In the light of these results they suggested therefore, that tocopherol has a quite specific role in the cholesterol metabolism.

The interrelationship of cholesterol and tocopherol has also been studied in humans. Darby and his co-workers (1949) in examining the plasma tocopherol levels in various pathological conditions, observed that in diseases with associated hypercholesterolemia there frequently were elevated plasma tocopherol values. Such conditions were, for example xanthomatosis, diabetes and hypercarotenemia Further they pointed out that hypertocopherolemia as well as hypercholesterolemia is often present during pregnancy and in cardiovascular diseases, whereas in conditions with low serum cholesterol values the tocopherol also was decreased. After demonstrating that this was not a methodological error they concluded that the explanation was an increased lipid-carrying power of the serum Attention was also paid by Popper and his group (1949) to the parallel behavior of cholesterol and tocopherol in diseases of the liver and the biliary tract. In diseases in which the excretion of cholesterol in the bile was impaired and retention into the

blood circulation occurred there was hypertocopherolemia. However contrary to the case with cholesterol, the tocopherol concentration in the bile did not rise above that in the serum. Klatskin and Krehl (1950) on the other hand, observed no correlation between the serum cholesterol and tocopherol in patients with hepatic cirrhosis.

The relationship between tocopherol and cholesterol in diabetes has been studied by Bensley and his co-workers (1950) and Vanzetti and his group (1956) The first mentioned observed a significant positive correlation between the plasma level of these two lipids and suggested that the plasma tocopherol content is directly dependent on the serum cholesterol level. On the other hand, no relationship was seen between the blood sugar and the serum tocopherol. Vanzetti *et al.* studied the interrelationship between cholesterol, total lipids and tocopherol in healthy aged persons and patients with arteriosclerosis (mean age 72 years) and in diabetics. No significant difference in the plasma tocopherol level was seen between the arteriosclerosis and control groups, whereas the mean tocopherol level in the diabetics was higher than in the healthy subjects and showed a good correlation to the cholesterol values.

In the study of Postel (1956) a positive correlation between the serum tocopherol and cholesterol levels in thyroid disorders was observed. He concluded that the thyroid hormone had a similar effect on the tocopherol and cholesterol metabolisms.

Tocopherol and Atherogenesis

The high peroxide number of the lipids in the wall of the atherosclerotic artery (Glavind *et al.* 1952) has given rise to a suspicion that tocopherol may be effective in preventing the formation of atheroma. The atherosclerotic aorta, however contains considerably more tocopherol than the normal aorta (Vannotti and Gervasoni 1957) It was stated by McCormick and McCluer (1960) that the amount of tocopherol present in the atheromatous aorta is sufficient to prevent the peroxidation of lipids. Weitzel (1957) in an extensive study of the antisclerotic effect of the fat-soluble vitamins, observed that vitamin E had only a low action in this respect.

Conflicting results have also been obtained in studies of the effect of large vitamin E doses on the serum lipids. Bronte-Stewart *et al.* (1956) and Beveridge *et al.* (1957) stated that the lowering action of unsaturated fatty acids on serum cholesterol was not due to their high tocopherol content. Greenblatt (1957) administered massive doses of vitamin E (40 gm of tocopherol per day) to 6 test subjects and observed the greatest decrease of serum cholesterol in persons with hypercholesterolemia, the drop being in some cases as much as 100 mg/100 ml. After the administration of 100 mg of alpha-tocopherol acetate during 12 days to healthy subjects, Gray and Loh (1958) recorded a significant increase in the serum cholesterol and phospholipid levels. Hammerl and Pichler (1960) effected a decrease in

the serum cholesterol level in atherosclerotic patients by combined treatment with vitamins A, E and K. In his monograph Pezold (1961) concluded that the results of animal and clinical experiments have been so contradictory that tocopherol deficiency or its administration in pharmacological doses apparently has no effect on atherogenesis.

OBJECT OF THE PRESENT INVESTIGATION

The present investigation is an attempt to throw further light on lipid metabolism in coronary heart disease.

In order to eliminate disturbances in the endogenous synthesis of lipids and thus facilitate the interpretation of the results, vitamins A and E, representing purely exogenous lipids, were chosen as the subjects of investigation. This seemed to be useful also for the reason that only few data are available of the serum tocopherol content or of the lipoprotein kinetics of the two vitamins in states of altered lipid metabolism. The serum cholesterol and triglyceride levels were also analyzed in order to classify the lipid disorder of each subject. In addition, the interrelationships of all these lipids may be of some interest.

The investigation is divided into five parts

1) Determination of the serum cholesterol, triglyceride and vitamin E and A levels in coronary and control subjects;
2) Interrelationship of the serum cholesterol, triglyceride, vitamin E and A levels in coronary and control subjects;
3) Influence of acute myocardial infarction on the serum cholesterol, triglyceride and vitamin E levels;
4) Vitamin E and A loading tests in coronary and control subjects;
5) Lipoprotein kinetics of vitamins E and A in coronary and control subjects.

The investigation was begun with the vitamin A studies. However vitamin A exhibited some disadvantages. The method for determining vitamin A in the plasma and particularly in the lipoprotein fractions was not fully adequate and, furthermore, side effects — nausea and headache — frequently occurred during the vitamin A loading tests. The vitamin A studies were therefore discontinued before the series of experiments was completed.

Some of the present data have been previously published as preliminary reports (Nikkilä and Pelkonen 1961, 1962 a, 1962 b, 1963)

MATERIAL

The series studied consisted of one group of 124 survivors of myocardial infarction and of two control groups, i.e., 322 blood donors and 101 healthy persons.

Survivors of myocardial infarction

This group included 110 male and 14 female patients between the ages of 30 to 65 years. All the patients in this group were admitted to the First or the Third Department of Medicine University of Helsinki, in 1960—1962 because of symptoms of acute myocardial infarction or angina pectoris. All had electrocardiographic changes unequivocally indicating an old or recent myocardial infarction.

Patients with diseases known to affect the lipid metabolism, such as diabetes mellitus and thyroid disorders, as well as patients with other severe diseases were excluded. Heart failure which may influence the fat absorption (Mäkelä *et al.* 1960) was also regarded as an excluding factor

During the acute stage of illness the patients were on a light caloric diet (1000 Cal. including 50 gm of fat per day) and thereafter on an ordinary hospital diet (1500 Cal., including 90 mg fat per day) The majority of the patients (100 of 124) were treated with oral anticoagulants.

The lipid analyses were made after a minimum of three weeks had passed from the acute stage of the illness. In the majority of cases they were done during the fifth week. The patients were then already mobilized from the bed

In the text to follow this group is briefly termed coronary

Blood donors

This group included 176 male and 146 female blood donors of the Blood Bank of the Finnish Red Cross. The age range was 20 to 65 years. No medical examination was performed with the exception of the routine hemoglobin determination. Donors who had taken any vitamin preparations within one week were excluded. The samples were taken without regard to the state of absorption and were analyzed even though the plasma was lactescent.

Healthy subjects

The test subjects in this group comprised 88 males and 13 females in the age range of 20 to 58 years.

The persons were either patients under medical observation in the First Department of Medicine University of Helsinki, or medical students working in the hospital.

The patients included in this group were admitted to the hospital for a cardiologic examination because of congenital heart disease without marked hemodynamic changes. The medical examination revealed no organic disorders in some patients and they were thus included in this group. Values of 350 mg/100 ml for serum cholesterol and of 500 mg/100 ml for serum triglyceride were regarded as criteria of hypercholesterolemic and hyperlipemic diseases. Persons who exceeded these lipid concentrations were excluded from this group. The medical students underwent no detailed medical examination but they were subjectively healthy

The medical students continued their customary dietary habits and the patients received the usual hospital diet (1500 Cal., including 90 gm fat per day)

In the following text this group is briefly termed healthy

METHODS

All samples, with the exception of those for plasma tocopherol and serum cholesterol analyses from the blood donors, were taken in the morning after an overnight fasting of 12 hours. In order to standardize the postabsorptive state, all the patients had one plate of porridge in the preceding evening 12 hours before the blood tap. Drinking of water was allowed during the fasting.

The plasma or serum was separated immediately after the blood was drawn, and was stored at +4 C The analyses were made within one week after the blood tap. The vitamin A determinations, however were done within two days.

All analyses were performed in duplicate. It the estimations of duplicates differed by more than 10 per cent, the samples were reanalyzed.

Serum Total Cholesterol

The serum total cholesterol was determined by the method of Pearson *et al.* (1953) When this method was compared with the method of Abell *et al.* (1952) it appeared that the latter gave cholesterol concentrations approximately 10 per cent lower irrespective of the cholesterol concentration (Nikkilä and Pelkonen 1963)

Serum Triglyceride

The serum triglyceride was determined by measurement of the glycerol component of the triglyceride molecule after saponification. The method used in this study was a combined modification of two methods (van Handel and Zilversmit 1957 Carlson and Wadström 1959) The extraction and purification of triglyceride was performed according to van Handel and Zilversmit with a mixture of chloroform and a zeolite (Doucil, W A. Taylor Company) since this procedure was simpler than the chromatographic method of Carlson and Wadström. To avoid the common occurrence of opalescence in the final step of the van Handel and Zilversmit procedure, the extraction of fatty acids with petroleum ether after saponification was included as in the method of Carlson and Wadström.

Plasma vitamin A

The plasma vitamin A determinations were made from heparinized

plasma, with the exception of the lipoprotein analyses, where Na-EDTA plasma was used. The different plasmas gave the same results when compared. A modification of the spectrophotometric method was used (Bessey *et al.* 1946)

After saponification with a mixture of absolute alcohol — potassium hydroxide, the vitamin was extracted with n-heptane. The extraction showed a recovery of 70—80 per cent even at the high plasma concentrations obtained during the vitamin A loading tests. The heptane extract was divided into two parts, one of which was irradiated in ultraviolet light to destroy the vitamin A. The difference in the optical densities of these two parts measured at wave length 328 mμ in the spectrophotometer (Beckman DU) thus showed the actual amount of vitamin A in the extract. However the optical densities of the irradiated extracts were so fixed at the level of 0.002—0.008 that the irradiation was discontinued. In making the calculations a value of 0.005 corresponding to the mean optical density of the irradiated extracts, was subtracted from the reading. All the samples were stored in complete darkness and direct daylight was avoided during the analysis procedure.

Plasma Tocopherol

The plasma tocopherol was determined after extraction with xylene by means of the dipyridyl-ferric chloride color reaction (Rindi 1957). Heparinized plasma was used, the lipoprotein tocopherol, however was estimated from the Na-EDTA plasma. Analyses of the different plasmas as well as of the serum showed no differences in the tocopherol concentration. The addition of cholesterol in increasing amounts (1.6—3.2—4.8 mg) into the extracts of tocopherol analyses did not alter the optical density readings at wave lengths 520 mμ.

Isolation of the Plasma Lipoproteins

The lipoprotein particles were fractionated according to their hydrated densities by flotation in a Spinco Model L preparative ultracentrifuge. Two runs were needed to obtain the following five particle classes: D < 1.006, D > 1.006, D 1.006—1.019, D 1.019—1.063, and D > 1.063 (Havel *et al.* 1955)

In the primary separation 5 ml Na-EDTA plasma was layered with a syringe and needle under an equal volume of 0.85 per cent sodium chloride solution in three lusteroid tubes with a volume of 13.5 ml. After a run in a rotor 40 for 30 minutes at a speed of 21,000 r.p.m. (28,360 x G) two layers were obtained. The upper turbid layer contained thus D < 1.006 particles and the lower clear layer D > 1.006 particles. From one of the tubes the two layers were separated for further chemical analysis. From the other two tubes the lower parts were removed by suction and placed in two empty tubes. To obtain the desired densities (D 1.019 and 1.063) equal volumes of 5.1 per cent sodium chloride solution

(D 1.032) was added to the one tube and 18.7 per cent sodium chloride solution (D 1.120) to the other tube The tubes were then run in the ultracentrifuge for 18 hours at a speed of 40 000 r p.m. (105 400 x G) Three layers were obtained. The turbid top layers contained the D 1.006—1.019 and D 1.006—1.063 particles, the water-clear color less middle layers were free of lipoproteins, and the yellow bottom layers consisted of D > 1.019 and D > 1.063 particles. Then 2 ml of the surface layers D < 1.019 and D < 1.063 and of the bottom layer D > 1.063 were again suctioned and distilled water was added to make the initial plasma volume of 5 ml. The fraction D 1.019—1.063 was calculated by subtracting the D 1.006—1 019 fraction from the D 1.006—1.063 fraction.

The concentration of vitamin A or tocopherol in lipoprotein fractions was determined. The method of vitamin A determination, however was not sensitive enough for lipoprotein analyses of fasting plasma. The recovery of vitamin A in the lipoprotein analyses showed a great variability in the range of 40 to 150 per cent. The samp.es where the recovery was less than 50 per cent or more than 125 pe cent were discarded.

The recovery of tocopherol in the lipoprotein analyses was, on the aver age, as high as 90 per cent in the primary separation and 80 per cent in the second separation. Here the samp.es were discarded if the recovery was less than 60 per cent or more than 125 per cent.

Vitamin A Loading Test

The vitamin A and E tests were identical in principle. At 8 a.m., after overnight fasting of 12 hours duration, the vitamin was given with 100 ml of cream containing 40 per cent fat. The total amounts of fat were 45.9 gm in the vitamin A loading tests and 49.2 gm in the vitamin E loading tests. The subjects continued to fast until the 6-hour blood sample was taken, after which they had the usual lunch. The 10-hour sample was taken after dinner On the next morning the last, or 24-hour sample was tapped, again preceded by overnight fasting.

During fasting, the ingestion of small amounts of water and smoking were allowed. Movement was limited to ambulation in the ward. The only ambulatory subjects included in this study were the medical students who underwent the vitamin A loading test.

To reach sufficiently high plasma concentrations for lipoprotein analyses rather large amounts of vitamin A was used. Thus 1.5 million I.U of v'tamin A palmitate in 5 ml of soyabean oil (A Vitol forte Orion) were given.

For the complete test, seven samples (at 0 2, 3, 4 6, 10 and 24 hours) were taken for the vitamin A determinations. Only the fasting and 24-hour samples were studied in the medical students, however The vitamin A con tent of lipoproteins was analyzed in the 4, 6, 10 and 24-hour samples.

In two subjects the vitamin A load ing test was done twice, and the results give some information of the reproducibility of the test (table I)

TABLE I. Plasma vitamin A level in two subjects during two successive vitamin A loading tests

Subjects	*Date*	*Fasting*	*2*	*3*	*4*	*6*	*10*	*24 hrs.*
Case 1 (healthy)	March 13, 1961	111	687	718	3857	2276	1319	421 I.U./100 ml.
	March 27 1961	128	887	2800	4356	4163	—	457
Case 2 (coronary)	Oct. 24, 1960	241	477	1022	2762	4000	3313	—
	Nov 10, 1960	267	326	—	3107	5375	5003	—

The above data show that in spite of a marked difference in the rate of absorption in the two tests with each of the subjects, the reproducibility of the test increased toward the end of the curve.

Tocopherol Loading Test

Two grams of alpha tocopherol acetate in 10 ml arachidis oil (Evitol, Orion) was given with 100 ml of cream (40 per cent fat) No side effects were observed during this test.

The blood samples were taken in the complete test 4, 6, 10 24 hours after ingestion of vitamin E. Because of the limited laboratory capacity only the 0 4, and 24-hour or the 0 and 24-hour samples were taken from some subjects. The complete lipoprotein analyses were made from the 0 4 6 10 and 24-hour samples.

The ordinary hospital meal had no influence on the plasma tocopherol content studied in 5 subjects. The loading test without tocopherol supplement was also performed in 5 subjects and no changes in the plasma tocopherol content were observed.

In order to study further the reproducibility the tocopherol test was per formed twice in one patient with hyperlipemia who showed a very abnormal response and the following results were obtained (table II)

Here again the best reproducibility was obtained at 24 hours.

In both the loading tests the serum *triglyceride levels* as well as the triglyceride content of the lipoproteins were determined, but will be published elsewhere

Statistical Procedures

The mean values presented are the arithmetic means calculated from the individual *observations and* are com pared by means of the Student s *t* test.

TABLE II. Plasma tocopherol level in patient with hyperlipemia during two successive tocopherol loading tests

Patient with hyper lipemia	*Fasting*	*4*	*6*	*10*	*24 hrs.*
Sept. 15, 1961	28.9	88.4	118.2	231.9	108.5 mg/L
June 12, 1962	45 1	78.1	83 6	123.5	110.1 mg/L

In the correlation studies, linear regression analyses were made and the correlation coefficient of Pearson was used, with the exception of the vitamin A studies, in which Spearman's rank order correlation coefficient (r_S) was used. The significance of the correlations was tested by the test variable

$$t = \sqrt{n-2}\,\frac{r}{\sqrt{1-r^2}}$$

For the statistical calculations employed the reader is referred to the monography of Walker and Lev (1953)

RESULTS

Since all the control series are not comparable with the coronary series with respect to age, they were divided into age groups below and over the age of 35 years. This age limit was chosen because according to autopsy data, the incidence of atherosclerosis increases sharply after the 35th year. Thus, the younger age group best represents a population free from active atherosclerosis. The coronary group was also divided into two age groups below and over 50 years, because the various degenerative processes associated with aging become more apparent after this age. The younger coronary group is therefore best suited for the study of a possible metabolic defect.

SERUM TOTAL CHOLESTEROL LEVEL

Blood Donors and Healthy Subjects

The serum cholesterol content was determined in 312 blood donors (mean age 41 years) 175 of whom were males (mean age 39 years) and 137 were females (mean age 44 years) and in 101 healthy subjects (mean age 31 years) The healthy group consisted of 85 men (mean age 32 years) and 16 women (mean age 26 years) The data are given in tables 1 and 2 as mean values with corresponding standard deviations, grouped according to the age of the subjects. Figure 1 depicts the influence of age. In addition, the cumulative frequency distribution curves of serum cholesterol in the blood donors are presented in fig 2.

The range of the serum cholesterol content in the male blood donors was from 145 to 438 mg/100 ml and in the female blood donors from 182 to 408 mg/100 ml. By definition true hyper cholesterolemic persons did not occur in the healthy group. The 350 mg/100 ml limit for true hypercholesterolemia was exceeded by 10 blood donors (3 per cent) 7 of whom were females and only one was younger than 35 years.

Comparison of healthy subjects and blood donors revealed a higher mean cholesterol content in blood donors. This, however was not unexpected since hypercholesterolemic subjects were excluded from the healthy group.

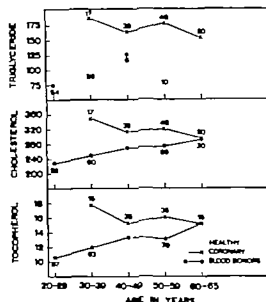


Fig. 1. Serum triglycerid (mg/100 ml) total cholesterol (mg/100 ml) and tocopherol (mg/L) levels in blood donors, healthy per sons and in survivors of myocardial infarc tion grouped according to age. Figures refer to number of subjects.

On the other hand, the age distribution was different. Two-thirds of the heal thy subjects were under 35 years of age, while three-fifths of the blood donors were in the older group. Accordingly the mean values in the two younger groups were almost identical.

A definite age trend was found (table 1 and fig. 1). The younger blood donors (age below 35 years) showed a significantly lower ($p < 0.001$) mean cholesterol content than the older ones (table 2 fig. 2). The mean cholesterol content in blood donors increased with each decade throughout the entire age range (table 1, fig. 1). The increase from the fifth to the sixth decade was, however only 2.5 mg/100 ml. This was due to the different age-cholesterol relationship between the two sexes. The men showed an increase up to the fifth decade, after which the cholesterol values began to decrease. Unfortunately the oldest male group (60—65 years) was too small to allow definite conclusions. On the other hand, the female cholesterol values continued to increase up to seventh decade.

Women showed a slightly higher mean cholesterol content than men, and the difference remained when the blood donor material was divided into the two age groups seen in table 2. This no longer was the case when the five decades were compared (table 1). Here the men of middle age showed higher mean value. The differences, however were hardly significant.

ABLE 1. Serum total cholesterol level (mg/100 ml) in 175 male and 137 female blood donors and 124 survivors of myocardial infarction, grouped according to age

Subjects	20—29 years			30—39 years			40—49 years			50—59 years			60—65 years		
	N	Mean	S.D	N.	Mean	S.D	N	Mean	S.D.	N.	Mean	S.D	N	Mean	S.D
lood donors															
males	56	226.2	50.4	34	251.1	43.2	35	275.6	52.0	41	268.6	48.0	6	256.2	34.4
females	25	236.2	31.7	26	249.8	45.1	33	263.9	52.4	28	280.3	46.3	24	300.5	47.2
total	82	229.3	45.4	60	250.6	42.6	71	271.0	50.6	69	273.5	47.3	30	291.7	46.8
oronary	—	—	—	17	348.2	125.9	39	312.7	68.9	48	319.0	91.9	20	298.4	60.1

TABLE 2. Serum total cholesterol level (mg/100 ml) in blood donors and healthy persons, in age groups below and over 35 years

Subjects	20—35 years			36—65 years			Total		
	N	Mean	S.D	N	Mean	S.D.	N.	Mean	S.D
Blood donors									
males	85	234.6	43.0	90	269.9	47.4	175	252.8	51.5
females	45	241.6	40.6	92	278.3	48.5	137	266.2	49.0
total	130	237.0	44.6	182	274.1	48.0	312	258.7	50.8
Healthy	67	233.6	33.9	34	244.5	40.6	101	237.3	36.5

Survivors of Myocardial Infarction

The serum cholesterol content was determined in 114 male (mean age 50 years) and 10 female survivors (mean age 51 years) of myocardial infarction. The mean age of the whole coronary group was 50 years. The data are given in tables 1 and 3 as mean values with standard deviations, grouped according to the age of the patient. Fig. 1 shows the influence of age, and in fig. 2 the cumulative frequency distribution of the serum cholesterol values is presented.

The range of cholesterol values in men was from 185 to 810 mg/100 ml and in women from 207 to 502 mg/100 ml. The female patients showed a considerably higher mean content than the male patients (table 3) but the female group was too small for statistical treatment. The frequency of true hypercholesterolemia, when the lower limit was defined as 350 mg/100 ml, was about 25 per cent, two-thirds of these 31 patients were under 50 years of age. The *younger* patients, i.e. those under age 50 showed a higher mean content than the older patients (table 3) but the difference was not significant. When the patients were divided according to age in decades (table 1, fig. 1) the age dependence became more apparent. Definite decrease occurred from the fourth to the fifth and from the sixth to the seventh decade.

The coronary patients showed a significantly higher ($p < 0.001$) mean serum content of cholesterol than the blood donors. The age distribution as well as the age-cholesterol relationship were different, as seen in fig. 1. However the mean levels in coronary patients were significantly higher than those of blood donors in each decade

TABLE 3. Serum total cholesterol level (mg/100 ml) in survivors of myocardial infarction, in age groups below and over 50 years

Subjects	30—50 years			51—65 years			Total		
	N	Mean	S.D.	N	Mean	S.D	N.	Mean	S.D
Coronary									
males	60	326.6	106.5	54	300.9	87.4	114	314.4	97.4
females	4	—	—	6	—	—	10	351.1	74.6
total	64	330.6	106.3	60	303.2	86.6	124	317.4	96.8

(p < 0 01) up to the seventh decade when they were nearly identical. The female blood donors showed in seventh decade even a higher mean content than the coronary patients.

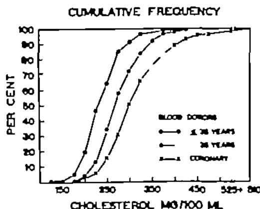


Fig. 2. Cumulative frequency distribution of serum total cholesterol in 130 blood donors below and 182 blood donors over the age of 35, and in 124 survivors of myocardial infarction.

In order to study the effectiveness of the various lipid parameters in separat ing the normal population from the diseased the 90 per cent upper limit of the younger blood donors (age below 35 years) was used as discriminator As is seen in the cumulative frequency distribution curves (fig. 2) this limit for cholesterol was 290 mg/100 ml.

Of the healthy subjects, 4 persons in both age groups (table 2) exceeded this limit, making 6 and 12 per cent. On the other hand, 58 older blood don ors (age over 35 years) (31 per cent) were above this limit (fig. 2) Of the coronary patients, 60 per cent showed higher values. This percentage was 70 per cent for the younger patients (age below 50 years) and only 50 per cent for the older patients.

SERUM TRIGLYCERIDE LEVEL

Healthy Subjects

The serum triglyceride was deter mined in 84 healthy men (mean age 32 years) and in 14 healthy women (mean age 27 years) The mean age of the total healthy group was 31 years.

The data are given in tables 4 and 5 as mean values with standard devia tions, grouped according to the age of the subjects. Fig. 1 shows the influence of age, and the cumulative frequency distribution of the serum triglyceride values is seen in fig. 3

TABLE 4. Serum triglyceride level (mg/100 ml) in 98 healthy persons and 124 survivors of myoca dial infarction, grouped according to age

Subjects	20—29 years			30—39 years			40—49 years			50—59 years			60—63 years		
	N	Mean	S.D	N	Mean	S.D	N	Mean	S.D.	N.	Mean	S.D	N.	Mean	S.
Healthy	54	73.0	34.8	28	101.9	91.6	6	129 7	56.1	10	90 4	32.4	—	—	—
Coronary	—	—	—	17	187.8	84.8	39	163 4	73.4	48	177.9	90.8	20	154.3	69

TABLE 5. Serum triglyceride level (mg/100 ml) in healthy subjects, in age groups below and over 35 years

Subjects	20—35 years			36—65 years			Total		
	N	Mean	S.D.	N.	Mean	S.D.	N	Mean	S.D.
Healthy									
males	56	79.8	33.4	28	107.1	39.9	84	88.4	37.9
females	11	—	—	3	—	—	14	83.1	36.8
total	67	79.1	33.9	31	105.8	39.1	98	87.6	37.4

In men the triglyceride levels ranged from 32 to 188 mg/100 ml and in women from 41 to 152 mg/100 ml. The small group of females thus had an almost identical mean content as the males (table 5) Because of the unequal age distribution, as seen in fig. 1, it is difficult to evaluate the influence of age on the serum triglyceride content. However the older subjects over the age of 35 years (table 5) showed a significantly higher mean content than the young persons ($p < 0.01$)

Survivors of Myocardial Infarction

The serum triglyceride content was determined in 114 male (mean age 50 years) and in 10 female (mean age 51 years) survivors of myocardial infarction. The mean age of the total group was 50 years.

The data are presented in tables 4 and 6 as mean values with corresponding standard deviations, grouped according to the age of the patients. The relation to age is shown in fig. 1, and the cumulative frequency distribution of serum triglyceride is seen in fig. 3.

The range of serum triglyceride values was from 63 to 453 mg/100 ml in men and from 78 to 322 mg/100 ml in women. The mean level in the younger patients (age below 50) was higher than that in the older patients, but the difference was not significant (table 6) When the patients were grouped by age decades (fig. 1 and table 4) the mean values of the groups showed a tendency to decrease with increasing age. The small group of female patients had a higher mean serum triglyceride content than the men (table 6)

The coronary patients had, on an average, significantly more serum triglyceride (tables 4, 5 and 6) than the healthy subjects ($p < 0.001$) How-

TABLE 6. Serum triglyceride level (mg/100 ml) in survivors of myocardial infarction, in age groups below and over 50 years

Subjects	30—50 years			51—65 years			Total		
	N	Mean	S.D	N.	Mean	S.D	N	Mean	S.D.
Coronary									
males	60	182.0	83.6	54	158.8	76.1	114	170.6	79.1
females	4	—	—	6	—	—	10	181.6	90.0
total	64	180.3	83.8	60	162.1	79.5	124	171.5	81.8

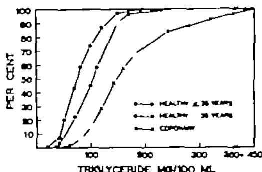


Fig. 2. Cumulative frequency distribution of serum fasting triglycerid in 67 healthy persons below and 31 healthy persons over the age of 35, and in 124 survivors of myocardial infarction.

ever as is seen in table 4 the age distribution was quite different. When the various age groups were compared the difference was nevertheless highly significant ($p < 0.001$) in the fourth and sixth decades. On the other hand, no significant difference was seen in the fifth decade but this age group consisted of 6 healthy persons only

The 90 per cent upper limit for the young healthy persons revealed a serum triglyceride content of 125 mg/100 ml (fig. 3) Thirty per cent of the older healthy persons (age over 35 years) exceeded this limit and the same percentage of the younger coronary group (age below 50 years) was below the limit, while 65 per cent of the older coronary patients exceeded it. The percentage of the whole coronary group was 68 per cent.

PLASMA TOCOPHEROL LEVEL

Blood Donors and Healthy Subjects

The plasma tocopherol content was determined in 176 male (mean age 39 years) and 146 female (mean age 43 years) blood donors, and in 62 healthy men (mean age 33 years) and 10 healthy women (mean age 26 years) The mean age of the total blood donor group was 41 years and that of the total healthy group 32 years.

The data are given in tables 7 and 8 and in the figure 1 as mean values with standard deviations. Fig. 4 depicts the cumulative frequency distribution of the plasma tocopherol values in the blood donor series.

ABLE 7 Plasma tocopherol level (mg/L) in 176 male and 146 female blood donors and in 100 urvivors of myocardial infarction, grouped according to age

Subjects	20—29 years			30—39 years			40—49 years			50—59 years			60—65 years		
	N.	Mean	S.D.	N	Mean	S.D	N.	Mean	S.D	N.	Mean	S.D	N.	Mean	S.D
Blood donors															
males	56	10.1	2.7	34	11.7	3.0	38	12.8	3.7	42	12.9	3.1	6	12.6	2.8
females	31	11.5	2.0	29	12.1	2.7	33	13.9	3.4	28	13.5	3.6	25	15.7	3.4
total	87	10.6	2.5	63	11.9	2.9	71	13.3	3.6	70	13.1	3.5	31	15.1	3.5
Coronary	—	—	—	15	17.7	3.9	35	15.2	3.4	35	16.1	3.6	15	15.1	2.9

TABLE 8. Plasma tocopherol level (mg/L) in blood donors and healthy persons, in age groups below and over 35 years

Subjects	20—35 years N	Mean	S.D	36—65 years N	Mean	S.D	Total N	Mean	S.D
Blood donors									
males	85	10.7	3.0	91	12.8	3.3	176	11.8	3.3
females	53	11.6	2.5	93	14.2	3.8	146	13.3	3.5
total	138	11.8	2.8	184	13.6	3.5	322	12.4	3.5
Healthy	48	10.5	2.1	24	11.1	2.5	72	10.7	2.3

The plasma tocopherol values of the male blood donors ranged from 6.1 to 23.3 mg/L, of the female blood donors from 6.9 to 23.6 mg/L, of the healthy males from 6.6 to 16.5 mg/L, and of the healthy women from 6.8 to 15.6 mg/L. The healthy subjects had, on an average, a lower plasma tocopherol content than the blood donors, but the age distribution was different. Thus, no difference existed in the younger groups (age below 35 years) as is seen in table 8. The age dependence was strikingly similar to that of serum cholesterol in fig. 1. No increase occurred, however in the female blood donors from the fifth to the sixth decade thus differing from the cholesterol-age relation. The men showed a decrease after the fifth or sixth decade also here (table 7) Independent of the age, the females had slightly higher mean values than the men (tables 7 and 8)

Survivors of Myocardial Infarction

The plasma tocopherol content was determined in 91 male (mean age 49 years) and 9 female (mean age 50 years) survivors of myocardial infarction, the mean age of the total group being 49 years.

The data are presented as mean values with standard deviations in tables 7 and 9 and in fig. 1. The cumulative frequency distribution of the plasma tocopherol values is given in fig. 4

The range of plasma tocopherol values was from 8.2 to 26.2 mg/L in males and from 8.9 to 20.4 mg/L in females. The women had, on an average, a slightly higher plasma tocopherol level than the men, but the number of women was too small for statistical treatment (table 9) The young patients showed a higher mean

TABLE 9 Plasma tocopherol level (mg/L) in survivors of myocardial infarction, in age groups below and over 50 years

Subjects	30—50 years N.	Mean	S.D	51—65 years N.	Mean	S.D	Total N	Mean	S.D
Coronary									
males	52	16.2	3.9	39	15.3	2.8	91	15.8	3.5
females	4	—	—	5	—	—	9	16.8	3.3
total	56	16.2	3.9	44	15.4	2.9	100	15.9	3.1

content than the old patients (age over 50 years) but the difference was not significant (table 9) Fig. 1 shows the similar age relations of the plasma tocopherol and the serum cholesterol values in the coronary population, revealing a definite tendency of plasma tocopherol content to decrease with age.

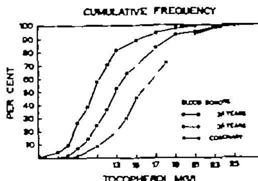


Fig. 4. Cumulative frequency distribution of plasma tocopherol in 118 blood donors below and 184 blood donors over the age of 35, and in 100 survivors of myocardial infarction.

On an average the coronary patients had more plasma tocopherol ($p < 0.001$) than the blood donors (tables 7 8 and 9) The difference was also statistically significant in all but the oldest decade (table 7 and fig. 1) where the mean contents were identical ($p < 0.001$ in fourth and sixth decades, $p < 0.01$ in the fifth decade)

The 90 per cent upper limit for the younger blood donors (age below 35 years) was 15 mg/L, as is seen in the cumulative frequency distribution curve (fig 4) Only one of the younger (age below 35 years) and 2 of the older healthy group exceeded this limit, but not less than 30 per cent of the older blood donors had higher values. On the other hand, 53 per cent of the coronary patients were above this limit, i.e., 55 per cent of the younger (age below 50 years) and 50 per cent of the older patients.

INTERRELATIONSHIPS OF SERUM TOTAL CHOLESTEROL, TRIGLYCERIDE AND TOCOPHEROL LEVELS

The correlation between the serum levels of the three lipids was studied by linear regression analysis and is shown in scattergrams in figs. 5—10 The regression equations and the correlation coefficients are presented in the legends to the figures.

A significant correlation ($p < 0.001$) was found to be present between the serum cholesterol and plasma tocopherol levels both in the coronary group and in the combined control group consisting of the healthy subjects and the blood donors. The regressions showed a somewhat greater increase of the serum cholesterol content with increasing tocopherol levels in the coronary group than in the control group (figs. 5 and 6)

On the other hand, a significant correlation ($p < 0.001$) between the serum triglyceride and plasma tocopherol levels was present only in the coronary group (figs. 7 and 8)

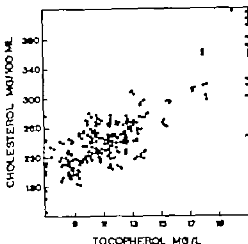


Fig 5 Relationship of serum cholesterol and plasma tocopherol levels in 312 blood donors and 68 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation. y = 122.4 + 10.94 x ± 32.6 (p < 0.001) = 0.75 (p < 0.001)

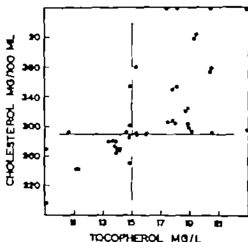


Fig. 6. Relationship of serum cholesterol and plasma tocopherol levels in 100 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 35 Lines = upper normal limits. Regression equation. y = 54.0 + 16.63 x ± 68.3 (p < 0.001) r = 0.65 (p < 0.001)

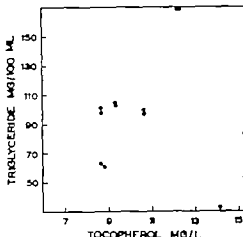


Fig 7 Relationship of serum triglyceride and plasma tocopherol levels in 68 healthy persons. Open dots = age below 35, black dots = age over 35.

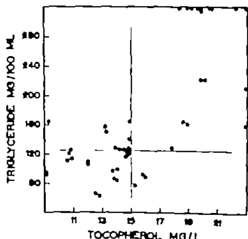


Fig 8. Relationship of serum triglyceride and plasma tocopherol levels in 100 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation. y = −70.5 + 15.25 x ± 68.3 (p < 0.001), = 0.63 (p < 0.001)

The regression analyses revealed a significant correlation between the serum triglyceride and cholesterol levels (figs. 9 and 10) in the coronary group ($p < 0.001$) and in the healthy group ($p < 0.05$). The regression line in both groups had a parallel course but in the coronary group it had a higher level, indicating a higher triglyceride content at the same cholesterol content.

The serum cholesterol, triglyceride and tocopherol contents were determined in 100 coronary patients. All the values were abnormally high in 44 cent and normal in 17 per cent. At least two abnormal lipid values were found in 56 per cent of the patients, while 27 per cent exhibited only one abnormal value. If only one lipid parameter was pathologic, this was most commonly the serum triglyceride (in 14 per cent of patients) and least commonly the plasma tocopherol (in 5 per cent).

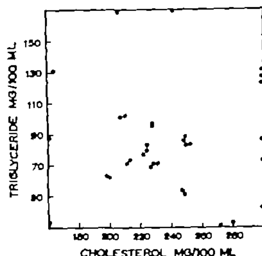


Fig. 9. Relationship of serum triglycerid and cholesterol levels in 94 healthy persons. Open dots = age below 35 years, black dots = age over 35. Regression equation: $y = 22.8 + 0.2507\,x \pm 37.9$ ($p < 0.05$) = 0.23 ($p < 0.05$)

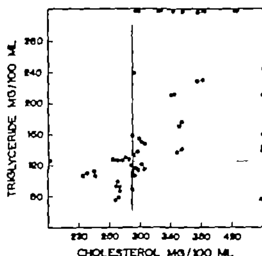


Fig. 10. Relationship of serum triglycerid and cholesterol levels in 124 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation. $y = 76.6 + 0.2901\,x \pm 77.8$ ($p < 0.001$) = 0.32 ($p < 0.001$)

INFLUENCE OF ACUTE MYOCARDIAL INFARCTION ON SERUM CHOLESTEROL, TRIGLYCERIDE AND TOCOPHEROL LEVELS

Numerous reports have been published of the changes occurring in the plasma lipids, lipoproteins and electrophoretic pattern after acute myocardial infarction (Welin 1948, Kroetz and Fischer 1954, Haus and Böhle 1955, Björck *et al.* 1957, Smith 1957, Dodds and Mills 1959, Page and Lewis 1959, Pomerantz 1962). However the data are somewhat controversial.

In order to study the influence of acute myocardial infarction on the plasma tocopherol, serum triglyceride and serum cholesterol levels, these values were followed in 9 patients until five months at least had elapsed from the acute attack. The first sample was taken within 24 hours after the patient s admission to this hospital. All the patients showed definite electrocardiographic changes due to the acute myocardial infarction and an increased serum activity of G—O-transaminase and lactic acid dehydrogenase at that time. The following three samples were taken at intervals of one week, and the last sample under ambulatory conditions when 5 to 11 months had elapsed from the acute infarction.

On admission an oral anticoagulant therapy was instituted in all cases, but none of the patients was given heparin. All but one patient were receiving anticoagulant therapy at the time the last sample was taken. No marked changes occurred in the dietary habits during the time of observation.

The mean tocopherol, triglyceride and cholesterol levels at each of the five dates are presented in fig. 11.

A significant fall ($p < 0.01$) occurred in the mean cholesterol and tocopherol levels during the first week. This was apparent in all the patients. On the other hand, no systematic change occurred in the triglyceride level. In one-half of the patients the triglyceride level decreased, while the other half showed an increase

Two weeks after admission the plasma tocopherol already reached the initial level, and under ambulatory conditions the plasma tocopherol and serum cholesterol showed higher though not significantly higher mean values than in the acute phase. The tocopherol mean at this time was the sum of an increase of greater magnitude in 5 patients than the decrease that occurred in 4 patients. On the other hand, all but one patient showed a higher cholesterol content during ambulation than on admission. The similar behavio of these two lipids after the infarction was striking.

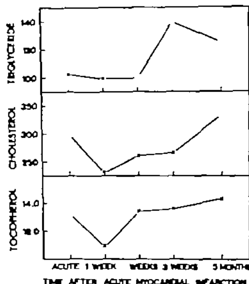


Fig. 11. Influence of acute myocardial infarction on serum cholesterol (mg/100 ml), triglyceride (mg/100 ml) and tocopherol (mg/L) levels in 9 patients.

The serum triglyceride level also was, on an average higher at the last examination than on admission. This was due to a definite increase in 6 patients, while the other 3 patients showed a negligible decrease. The high mean level in the third week was chiefly due to an unexpectedly high value in one patient.

TOCOPHEROL LOADING TEST

Two grams of tocopherol acetate and 100 ml of cream (40 per cent fat) was given under hospital conditions to 64 survivors of myocardial infarction (mean age 48 years) and to 38 healthy subjects (mean age 35 years) Six female coronary patients and three healthy females were included in the two groups. The coronary and healthy groups were divided into two categories according to age The age border line for coronary patients was 50 years and for healthy persons 35 years. The mean ages for the two coronary groups were 43 and 57 years and for the two healthy groups 26 and 45 years.

Table 10 presents the mean values at each time, with standard deviations. Fig. 12 shows the individual values and fig. 13 the absolute increase from the basal level of plasma tocopherol after the ingestion of vitamin E.

The complete loading test concerned five samples (basal sample and at 4, 6, 10 and 24 hours) but as is seen in the table 10 all the samples were not examined at each time of sampling This concerns particularly the 6- and 10-hour samples in the coronary group.

The basal levels of serum cholesterol and triglyceride were determined in all subjects. When the criteria for normal limits were those presented above these measurements revealed in coronary patients who underwent the test a hypercholesterolemia incidence of 73 per cent, while 75 per cent showed hypertriglyceridemia, and 61 per cent hypertocopherolemia. Thus, hyperlipidemia was somewhat more common in these subjects than in the whole series in this study With respect to lipid values the healthy subjects were comparable to the base population of this study with the exception of the older healthy subjects (age over 35 years) who showed hypertriglyceridemia in 39 per cent of subjects.

Fasting samples. — The coronary patients had a significantly higher mean tocopherol level ($p < 0.001$) than the healthy persons. The difference was highly significant also when the mean levels of the older healthy group and the younger coronary group were compared ($p < 0.001$)

4-hour samples. — The mean tocopherol level of the coronary group was still significantly higher than the mean level of the healthy subjects. However there was considerable overlapping of the individual values in the two populations. The younger subjects in both groups showed higher mean values than the older subjects due to more rapid mean increases. On the average the whole healthy group showed a slightly higher increase from the basal level, but statistically this was not significant.

6-hour samples. — The difference between the mean levels of the two groups was greater than in the 4-hour samples. The mean increase of the plasma tocopherol from the basal level was now greater in the coronary group than in healthy subjects, but it was not yet statistically significant. In both

groups the mean tocopherol content as well as the mean increase from the basal level still tended to remain higher in the younger subjects than in the older ones.

10-hour samples. — This value represented the peak level in all the subjects. Despite the significant difference in the mean values of coronary patients and healthy subjects, there was still marked overlapping of individual values in the two groups. The mean tocopherol level —1 S.D in coronary patients was exceeded by 31 per cent of healthy subjects. On the other hand, 28 per cent of the coronary patients had values below the mean +1 S.D of the healthy group. The older group of healthy subjects now showed a slightly higher mean content than the younger healthy group, while in the coronary group the two age categories had almost identical mean values. The mean increase from the basal level was now significantly higher in the coronary than in the healthy population ($p < 0.01$)

Both groups exhibited a significant correlation between the basal tocopherol level and the 10-hour peak level. The correlation coefficient in the coronary group was 0.63 ($p < 0.001$) and in the healthy group 0.41 ($p < 0.02$)

24-hour samples — The difference of the mean levels in the two main groups was significant at 24 hours ($p < 0.001$) The difference between the older healthy group and the younger coronary group was also statistically highly significant ($p < 0.001$) Nevertheless, a considerable overlapping of the individual values of coronary and healthy subjects was present. Thus 27 per cent of the healthy subjects exceeded the mean level —1 S.D of the coronary patients, and 24 per cent of the coronary patients were below the mean level +1 S.D of the healthy subjects. The older group of healthy subjects had a higher mean level than the younger group due to a significantly greater increase from the basal level ($p < 0.05$) Among the coronary patients the situation was the reverse the younger coronary subjects

TABLE 10. Plasma tocopherol level (mg/L) in healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat)

Subjects	Fasting			4 hours			6 hours			10 hours			24 hours		
	N.	Mean	S.D	N.	Mean	S.D	N	Mean	S.D.	N.	Mean	S.D	N	Mean	S.D.
Healthy															
age below 35	20	10.8	1.9	20	18.5	4.5	20	22.0	5.4	18	31.2	7.7	20	19.5	3.3
age over 35	18	11.2	2.6	17	16.6	5.2	16	21.1	7.6	17	34.0	10.3	18	22.6	5.2
total	38	11.0	2.3	37	17.6	4.8	36	21.6	6.4	35	32.6	9.0	38	21.0	4.5
Coronary															
age below 50	41	16.7	4.0	40	23.7	6.3	24	31.1	7.7	25	45.6	11.3	40	31.9	7.9
age over 50	23	16.4	3.0	22	20.7	4.4	7	27.1	6.4	7	48.2	12.3	23	28.5	8.2
total	64	16.6	3.6	62	22.7	5.9	31	30.2	7.5	32	45.8	11.3	63	30.7	7.5

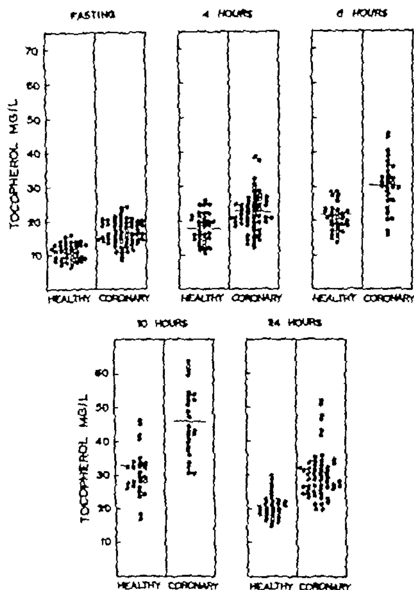


Fig. 12. Plasma tocopherol levels in healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat). Healthy: Open dots = age below 35, black dots = age over 35. Coronary: Open dots = age below 50, black dots = age over 50. Horizontal lines = mean tocopherol levels.

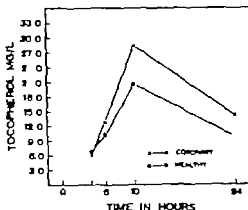


Fig. 13. Absolute increase of plasma tocopherol content from the basal level in 33 healthy persons and 63 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat)

having a higher mean level than the older patients and the increase from the basal level also being higher ($p < 0.05$) The mean increase from the basal level remained significantly higher in the coronary group than in the healthy group ($p < 0.02$)

The increase of plasma tocopherol content from the basal level was continuous up to the peak level in all the subjects with the exception of two who showed no increase in the interval from 4 to 6 hours, and one, who showed no increase from the basal level in the 4-hour sample In all the subjects the 24-hour level was higher than the basal level but lower than the 10-hour level.

The plasma tocopherol concentration curve calculated on the basis of samples taken 4, 6, 10 and 24 hours after the ingestion of tocopherol was thus significantly higher in the coronary patients than in the somewhat younger healthy group. Considerable overlapping of individual values was present at each time, the least in the 24-hour samples. Because of a different age distribution of the two groups, the data are not readily comparable The mean ages and age distributions, however were identical in the older healthy group and in the younger coronary group. These groups are therefore the best suited for comparison. The results in these groups revealed a similar significant difference as the healthy and coronary groups as a whole.

An abnormal retention of tocopherol at 24 hours may reflect some defect in the lipid metabolism, particularly in the disappearance of exogenic lipids from the circulation. The relation of the level at 24 hours to the basal level of tocopherol and to those of triglyceride and cholesterol, the two important constituents of plasma lipids, was therefore studied by linear regression analysis. The relationship of these lipid parameters is demonstrated by plotting the individual values in scatter grams (figs. 14—19) The regression equations and correlation coefficients are presented in the legends to figs. 14—19 The analyses revealed a significant correlation between the 24-hour tocopherol level and the basal lipid level in both the healthy and the coronary groups, thus showing that no one of these parameters was an independent variable.

On the basis of this consideration it seemed to be useful to study the validity of the 24-hour tocopherol value in

separating the coronary population from the normal population, as compared to the validity of the basal level of plasma tocopherol, serum triglyceride and serum cholesterol. The 90 per cent upper limit of the young healthy subjects was used as a discriminator. In the 24-hour tocopherol determination this was 24.5 mg/L. Six persons in the older healthy group exceeded this limit, equivalent to 33 per cent. This percentage thus was almost the same as the incidence of hypertriglyceridemia. On the other hand, 83 per cent of the coronary patients exceeded this value, while the corresponding percentage of basal cholesterol, triglyceride and tocopherol were 73, 75 and 61 per cent. Accordingly the metabolic abnormality was best revealed by the loading test.

Comparison of the values of all these lipid parameters in the coronary group showed that all the parameters were abnormally high in 51 per cent of cases and within normal limits in 3 cases only. The 24-hour tocopherol level only was pathologic in 2 cases, but it was never normal alone.

It has been suggested that oral anticoagulants impair the removal of circulating fat in postprandial lipemia (Mashford and Nestel 1961). In the present series all but 9 coronary patients received oral anticoagulants. However, only one of these 9 subjects had a normal 24-hour plasma tocopherol level.

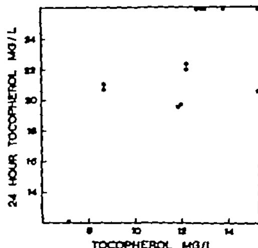


Fig. 14. Relationship of plasma 24-hour tocopherol and fasting tocopherol levels in 38 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation: $y = 6.95 + 1.275\,x \pm 3.49$ ($p < 0.001$), $r = 0.64$ ($p < 0.001$)

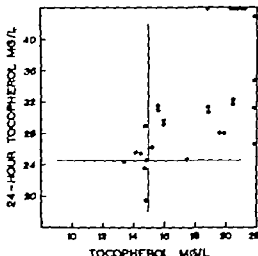


Fig. 15. Relationship of plasma 24-hour tocopherol and fasting tocopherol levels in 63 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation. $y = 7.15 + 1.420\,x \pm 5.46$ ($p < 0.001$), $r = 0.69$ ($p < 0.001$)

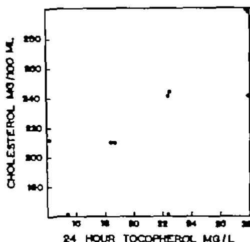


Fig. 16. Relationship of serum cholesterol and plasma 24-hour tocopherol levels in 38 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation. y = 140.4 + 4.619 x ± 33.3 (p < 0.001) = 0.54 (p < 0.001)

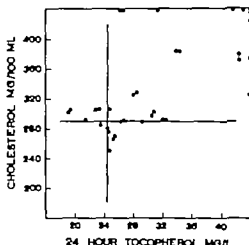


Fig. 17 Relationship of serum cholesterol and plasma 24-hour tocopherol levels in 63 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation. y = 86.1 + 8.212 x ± 81.8 (p < 0.001) = 0.60 (p < 0.001)

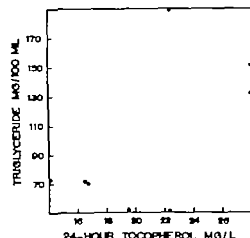


Fig 18. Relationship of serum fasting triglyceride and plasma 24-hour tocopherol levels in 38 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation: y = 19.5 + 4.149 x ± 26.9 (p < 0.01), r = 0.45 (p < 0.01)

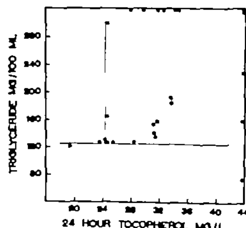


Fig. 19. Relationship of serum fasting triglyceride and plasma 24-hour tocopherol levels in 63 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation. y = 35.6 + 4.860 x ± 85.8 (p < 0.01) = 0.40 (p < 0.01)

TOCOPHEROL IN PLASMA LIPOPROTEINS DURING THE LOADING TEST

The lipoproteins were isolated from the plasma by two successive ultracentrifugal runs. In the primary separation fractions D < 1.006 and D > 1.006 were obtained. This separation was performed of fasting plasma samples and of samples taken 4, 6, 10 and 24 hours after the ingestion of 2 grams of tocopherol acetate and 100 ml of cream (40 per cent fat) by 17 survivors of myocardial infarction (mean age 47 years) and 25 healthy persons (mean age 33 years) The healthy group was divided according to age into two groups, the age borderline being 35 years. The mean ages of the younger healthy group was 27 years and of the older group 41 years. The subjects were men, with the exception of 3 women in the coronary group and 2 in the healthy group. The D > 1.006 fraction of 8 coronary patients (mean age 47 years) and of 7 healthy persons (mean age 33 years) was separated further into D 1.006—1.019 D 1.019—1.063 and in D > 1.063 fractions. This secondary separation was done of fasting plasma and of samples taken 4, 10 and 24 hours after the administration of tocopherol.

The results, expressed as mean values with standard deviations or ranges are shown in tables 11 and 12. The concentration curves for tocopherol in the fractions are presented in figs. 20 and 22 and the corresponding percentile

TABLE 11. Tocopherol content (mg/L) of D < 1.006 and D > 1.006 particles in sera of heal persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 of cream (40 per cent fat) Data corrected to 100 per cent recovery of total plasma content.

Subjects	D < 1.006 Fasting N.	Mean	S.D.	4 hours N	Mean	S.D.	6 hours N.	Mean	S.D.	10 hours N.	Mean	S.D	24 hours N	Mean	S
Healthy															
age below 35	15	1.9	1.3	10	6.0	4.1	12	5.0	2.9	12	7.0	4.7	14	2.9	
age over 35	8	1.5	0.9	8	5.0	2.4	7	4.6	1.8	11	8.4	3.7	11	3.9	
total	23	1.8	1.2	18	5.6	3.4	19	4.9	2.5	23	7.6	4.2	25	3.3	
Coronary	15	2.1	1.6	14	6.2	4.0	13	7.0	6.1	17	10.3	4.3	17	5.3	

Subjects	D. > 1.006 Fasting N.	Mean	S.D	4 hours N.	Mean	S.D	6 hours N	Mean	S.D.	10 hours N.	Mean	S.D.	24 hours N	Mean	S
Healthy															
age below 35	15	8.9	2.1	10	11.5	2.2	12	15.0	2.5	12	22.8	3.9	14	16.2	
age over 35	8	10.5	3.2	8	13.7	4.0	7	15.5	8.0	11	28.2	9.5	11	18.7	
total	23	9.4	2.6	18	12.5	3.2	19	15.2	3.5	23	24.4	7.2	25	17.4	
Coronary	15	15.9	3.1	14	19.1	4.8	13	25.2	6.8	17	37.0	8.9	17	29.3	

distributions in figs. 21 and 23. As will be seen in the tables, all the samples were not analyzed each time.

Serum cholesterol and fasting triglyceride were determined in all these subjects. Of the coronary patients all but 2 had hypercholesterolemia and all but 4 had hypertriglyceridemia. Only 2 coronary patients showed a normal tocopherol clearing at 24 hours, while in 6 healthy persons the 24-hour tocopherol level was abnormally high.

Tocopherol Content of D < 1.006 and D > 1.006 Particles

Fasting Samples. — Most of plasma tocopherol was found in the D > 1.006 particles. The mean ratio of D > 1.006 tocopherol to the total plasma tocopherol, expressed as per cent, was higher in coronary patients than in healthy persons, but the difference was not significant. The higher mean content of plasma tocopherol in the coronary patients as compared to the healthy subjects (calculated from table 11) was wholly accounted for by fraction D > 1.006, in which the tocopherol content was significantly higher in the coronary patients ($p < 0.001$). The ratio (D > 1.006/total plasma tocopherol) tended to be higher in older healthy persons than in the younger ones, but the groups were small.

Samples Taken 4, 6, 10 and 24 Hours after the Ingestion of Tocopherol. — In the early phase up to 4 hours the tocopherol content increased in both fractions, the increase being more rapid in D < 1.006 particles. *At 4 hours* the mean tocopherol content as well as the mean increase from the basal level in both particle classes were higher in the coronary than in the healthy

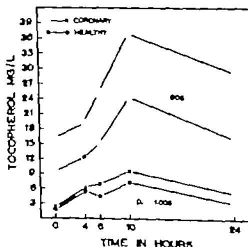


Fig. 20. Tocopherol content of D < 1.006 and D > 1.006 particles in sera of 25 healthy persons and 17 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat)

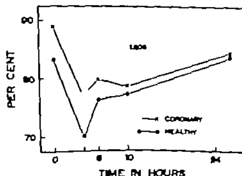


Fig. 21. Tocopherol content of D > 1.006 particles as per cent of total plasma content in 25 healthy persons and 17 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat)

group. The younger healthy subjects showed at 4 hours a higher mean content of D < 1.006 tocopherol and a lower content of D > 1.006 tocopherol than the older ones.

After 4 hours the D > 1.006 tocopherol showed a sharp and continuous increase in both main groups until the peak level was attained at 10 hours. The difference between the two main groups with respect to the tocopherol in this fraction increased with time until the peak level was reached and thus was significant at each time. Similarly the older healthy persons maintained, on the average, a higher content of D > 1.006 tocopherol up to the peak level at 10 hours, as compared to the young healthy persons.

The peak level of tocopherol in the light particles (D < 1.006) was also reached at 10 hours in both groups. The mean increase between 4 and 10 hours was, however considerably smaller than that of tocopherol in D $>$ 1.006 particles. Moreover the tocopherol content of the lighter particles decreased between 4 and 6 hours in the majority of healthy persons. During the same interval the increase in tocopherol in the D < 1.006 particles was the least apparent also in coronary population. Again, at each time the coronary group showed a higher mean content of tocopherol in the lighter particles than the healthy persons. The higher mean content of tocopherol in these particles observed up to 6 hours in the young healthy persons as compared to the older healthy persons was exceeded by old healthy persons at the peak level.

After the peak level D > 1.006 tocopherol showed in both groups a somewhat more rapid rate of decrease than the lighter particles. The rates of decrease of tocopherol in both particles was almost identical in the coronary and the healthy groups. At 24 hours none of the fractions had as yet declined to the initial level, and the tocopherol content of both fractions was higher in the coronary group than in the healthy group (D < 1.006 $p < 0.001$, D > 1.006 $p < 0.01$) The rate of decrease of the particulate tocopherol was almost identical in both healthy age groups. The tocopherol content of both fractions at 24 hours was somewhat higher in the older healthy persons as compared to the younger group.

Tocopherol Content of D 1.006—1.019 D 1.019—1.063 and D >1.063 Particles

Fasting Samples. — The mean tocopherol concentration in all subfractions with a density above 1.006 was higher in the coronary patients than in the healthy subjects. However the difference in the high density particles (D $>$ 1.063) was insignificant. Particles D 1.019—1.063 appeared to have the highest tocopherol content. In coronary patients, on the other hand, the tocopherol content of D 1.006—1.019 was only slightly lower than that of D 1.019—1.063 In both groups the high-density lipoproteins contained the least tocopherol

Samples Taken 4, 10 and 24 Hours after the Ingestion of Tocopherol. —

During the first hours the tocopherol content appeared to increase in all the fractions, the most in the D 1.006—1.019 fraction. *At 4 hours* the coronary group had a higher mean tocopherol content in D 1.006—1.019 and D 1.019—1.063 particles as compared to the healthy group, while the particles of highest density had an equal tocopherol content in the two groups due to a higher mean increase from the basal level in the healthy subjects. The peak level of tocopherol in the particle classes D 1.006—1.019 and D 1.019—1.063 was attained at 10 hours and was higher in coronary patients than in healthy subjects. From 4 to 10 hours the coronary group showed a higher mean increase of tocopherol in D 1.006—1.019 particles as compared to that in D 1.019—1.063 particles, while the situation was the opposite in healthy persons. The differences within the two groups were small, however.

After 10 hours D 1.006—1.019 tocopherol in both groups showed the most rapid decrease, the rate being slightly greater in the coronary than in the healthy group. Only a slight decrease of the tocopherol content of D 1.019—1.063 occurred between 10 and 24 hours in coronary patients, while the healthy persons showed a definite decrease. A difference between the two

TABLE 12. Tocopherol content (mg/L) of D 1.006—1.019, D 1.019—1.063 and D > 1.063 particles in sera of healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat). Data corrected to 100 per cent recovery of tocopherol content of D > 1.006 fraction.

Subjects	D. 1.006—1.019											
	Fasting			4 hours			10 hours			24 hours		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Healthy	5	3.4	2.6— 5.6	7	5.0	4.2— 6.7	7	11.3	3.6—22.0	7	5.3	3.8— 9.6
Coronary	6	7.6	4.7—11.8	8	9.2	6.9—11.6	6	20.4	12.2—30.9	6	13.6	8.8—19.4

Subjects	D 1.019—1.063											
	Fasting			4 hours			10 hours			24 hours		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Healthy	5	5.3	2.9— 7.0	7	6.2	2.4— 9.8	7	13.3	7.3—23.0	7	10.5	5.7—16.7
Coronary	6	8.1	6.3—12.8	8	8.5	1.0—11.9	6	18.5	10.8—33.6	6	17.6	9.6—31.6

Subjects	D > 1.063											
	Fasting			4 hours			10 hours			24 hours		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Healthy	5	1.5	1.4— 2.8	7	2.4	0.8— 5.7	7	3.1	2.4 5.6	7	3.1	1.8— 6.1
Coronary	6	1.8	1.5— 2.9	8	2.4	1.6— 4.6	6	3.4	0.4 4.4	6	4.5	0.4— 8.9

cant when the older healthy group and the younger coronary group are compared ($p < 0.02$) Overlapping of the individual values in the two groups of subjects was considerable Not less than 45 per cent of coronary patients fell below the mean content + 1 S.D of the healthy subjects, and the same proportion of healthy subjects exceeded the mean value —1 S.D of the coronary group. The 90 per cent upper limit of the young healthy subjects was 156 I.U./100 ml and was exceeded by 65 per cent of the coronary patients. No significant correlation was found between the plasma vitamin levels and serum triglyceride or cholesterol levels.

Samples at 2 3 and 4 hours. — All the subjects showed a continuous rise of the plasma vitamin A content from 0 to 4 hours. At each time the young healthy subjects had a higher mean content than the older healthy persons, the difference being the most marked at 3 hours. In the coronary group, on the other hand, the younger patients had a higher mean content than the older patients only at 2 hours, the difference was negligible at 3 hours, while at 4 hours the older patient group had a higher mean level. Comparison of the two main groups revealed that the increase from the basal level to 4 hours was on an average higher in the healthy group than in the coronary group. In spite of their lower initial mean content, the healthy subjects showed markedly higher

…LE 13. Plasma vitamin A level (I.U./100 ml) in healthy persons and survivors of myocar…
…tion after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per c…

…jects		Fasting N.	Fasting Mean	Fasting S.D	2 hours N	2 hours Mean	2 hours S.D	3 hours N.	3 hours Mean	3 hours S.D.	4 hours N.	4 hours Mean	S…
…thy													
… below	35	26	112	42	9	877	744	9	2064	1553	9	3123	2…
… over	35	10	131	45	8	869	577	8	1694	1041	9	3018	1…
…al		36	117	43	17	873	650	17	1890	1310	18	3071	1…
…nary													
… below	50	14	171	54	10	685	542	10	1478	1148	11	2382	1…
… over	50	20	165	45	19	510	432	19	1539	1400	18	2737	1…
…al		34	167	48	29	570	471	29	1517	1298	29	2603	1…

Subjects		6 hours N	6 hours Mean	6 hours S.D	10 hours N	10 hours Mean	10 hours S.D	24 hours N	24 hours Mean	S…
Healthy										
age below	35	9	3378	2353	10	1564	949	25	265	[illegible]
age over	35	9	2977	1399	10	1867	671	10	339	[illegible]
total		18	3177	1890	20	1716	864	35	303	[illegible]
Coronary										
age below	50	13	4828	1621	15	5248	3589	13	1134	1…
age over	50	19	4652	2059	18	3896	2271	16	739	[illegible]
total		32	4724	1867	33	4511	2973	29	917	[illegible]

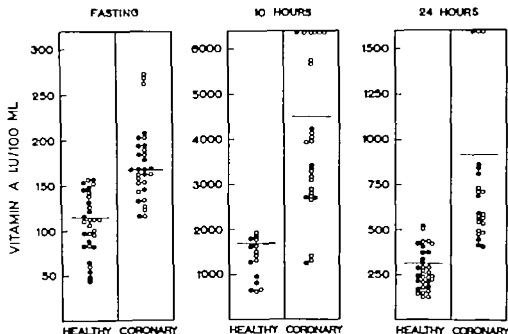


Fig. 24. Plasma vitamin A level in healthy persons and survivors of myocardial infarction at fasting stage and 10 hours and 24 hours after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat). Horizontal lines = mean vitamin A levels.

mean values at 4 hours. In 6 healthy subjects and in 2 coronary patients the 4-hour level was the peak level. A marked overlapping of the values between the two groups was present each time.

Samples at 6 Hours. — All but 2 healthy subjects reached the peak level in 6 hours. In the coronary group, on the other hand, 17 of the 28 patients (61 per cent) from whom a sufficient number of samples was examined to estimate the time of the peak level had it at 6 hours. However the mean peak level in both groups occurred at 6 hours. Of the healthy subjects the younger category still showed a higher mean level. The young coronary patients also showed a slightly higher mean level than the older ones. At 6 hours the mean level of the coronary group was above that of the healthy group ($p < 0.01$) though overlapping of the individual values was great.

Samples at 10 Hours. — Only two subjects in the healthy group showed a delayed peaking at 10 hours. They both had a normal fasting serum lipid pattern, including the vitamin A level. Delayed peaking occurred in the coronary group in 39 per cent. Only one of these patients was normocholesterolemic, three had normal triglyceride values, and not less than 7 of these 11 patients had a normal fasting vitamin A content.

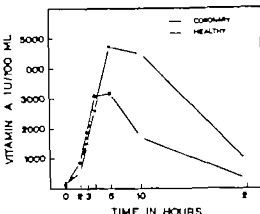


Fig. 25. Plasma vitamin A level in 36 healthy persons and 34 survivors of myocardial in farction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml f cream (40 per cent fat)

At 10 hours the older healthy per sons showed for the first time after the load, a higher mean level than the younger ones. The young coronary pati ents, on the contrary had a markedly higher mean content than the old patients. The coronary group had a significantly higher mean level ($p < 0.001$) than the healthy group and overlapping was no longer as marked as earlier as seen in the fig. 24.

Samples at 24 Hours — None of the subjects had reached the basal vitamin A level at 24 hours, but in all subjects the values were lower than at 10 hours. The difference in the mean levels of the two age categories of the healthy group was marked. In none of the older healthy subjects was the circulating vitamin A content at 24 hours below the mean level of the younger healthy subjects. The medical students showed a lower mean content (221 I.U /100 ml) than the hospital ized young healthy group (329 I.U 100 ml) The younger coronary group, on contrary had a markedly higher mean level than the older coronary group.

The plasma vitamin A retention at 24 hours was, on an average higher in coronary patients than in healthy subjects ($p < 0.001$) A significant dif ference ($p < 0.02$) existed also between the older healthy and the younger coronary groups. As seen in fig. 24 the overlapping of individual values of the two main groups was, however still present.

As was done in the tocopherol loading test, the 90 per cent upper limit of the vitamin A level at 24 hours in young healthy persons was calculated and was found to be 450 I.U /100 ml. This level was exceeded by 2 subjects in the older healthy group. A lower value at 24 hours was seen in only 3 patients in the older coronary group and in none of the younger patients. One of these normally clearing pa tients had a normal fasting lipid pat tern, another showed only hypertriglyceridemia, and the third had hyper vitaminosis A only

When the fasting plasma vitamin A levels and the 24-hour vitamin A levels were compared in each group the only significant correlation was found in the healthy group ($p < 0.01$) the correlation coefficient being 0.53 (fig. 26) Serum basal triglyceride levels were not in significant correlation with the plasma 24-hour vitamin A levels, but a significant correlation was found between serum basal cholesterol and the plasma 24-hour vitamin A levels

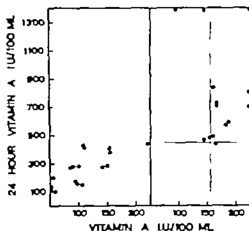


Fig. 28. Relationship of 24-hour plasma vitamin A and fasting vitamin A levels in (left) healthy persons and (right) 28 survivors of myocardial infarction. Healthy: Open dots = age below 35, black dots = age over 35. Coronary: Open dots = age below 50, black dots = age over 50. Lines = upper normal limits.

in the coronary group ($p < 0.001$) and in the healthy group ($p < 0.001$). The correlation coefficients were 0.63 and 0.55 respectively.

Thus the coronary and healthy groups handled the ingested vitamin A differently in many respects. It is, however, once again pointed out that these two groups were not comparable with respect to age. The older healthy group (age over 35 years) and th younger coronary group (age below 50 years) had, however, an equal mean ge and are best suited for comparison.

The healthy subjects showed a faster initial rise in the plasma vitamin A level. At 6 hours, but not before, the coronary patients had a higher mean plasma vitamin A content than th controls and thereafter the difference between the two groups became more apparent. The occurrence of the peak level varied from 4 to 10 hours. Regardless of the time the mean content at peak level in healthy subjects was 3466 I.U./100 ml (S.D. 1787), the mean of 3558 I.U./100 ml (S.D. 2403) in the younger healthy persons being higher than the 3385 I.U./100 ml (S.D. 1147) seen in the older healthy group. The coronary patients had a significantly higher ($p < 0.001$) plasma vitamin A peak content, 5899 I.U./100 ml (S.D. 2789) than the healthy group. The younger coronary patients had higher peak level, 6665 I.U./100 ml (S.D. 3189) than the older coronary patients, whose peak was 5324 I.U./100 ml (S.D. 2382).

On the whole the healthy subjects reached the peak level earlier or in other words, delayed peaking was more common in the coronary group. The peak level was attained at 10 hours by 11 of the 28 coronary patients but by only 2 of the 17 healthy subjects.

In 5 coronary subjects, all exhibiting a marked hypercholesterolemia and hypertriglyceridemia, both the vitamin A and the tocopherol loading tests were done. In both tests all the 5 subjects responded abnormally showing a high vitamin level at 24 hours. In addition, 4 of these subjects showed a delayed peaking in the vitamin A loading test.

Only 1 of the 8 patients who did not recieve oral anticoagulants had a normal 24-hour plasma vitamin A level, while the others showed an abnormally high vitamin A retention at 24 hours.

VITAMIN A IN PLASMA LIPOPROTEINS DURING THE LOADING TEST

Vitamin A was determined in five lipoprotein fractions which were isolated from plasma by two successive ultracentrifugal runs. In the primary separation, fractions D < 1 006 and D > 1 006 were obtained. This primary fractionation was performed from samples taken 4, 6 10 and 24 hours after ingestion of the vitamin in 12 survivors of myocardial infarction (mean age 52 years) and in 9 healthy men (mean age 53 years) One woman was included in the coronary group. The D > 1.006 fraction of 9 coronary patients (mean age 54 years) and of the 9 healthy men were separated further and in this secondary fractionation the D 1.006—1 019 D 1.019—1.063 and D > 1.063 fractions were obtained. The data are listed in tables 14 and 15 as mean values with standard deviations or ranges. Figs. 27 and 29 show the concentration curves for vitamin A in the different lipoproteins during the loading test. The percentile distribution of vitamin A in lipoproteins is presented in figs. 28 and 30 Because of technical difficulties the number of samples studied varied at each time as seen in the tables.

The serum cholesterol and triglyceride were studied in all the subjects. These measurements revealed that all but four of the coronary patients had hypercholesterolemia. Two of these 4 had also a normal triglyceride value. In addition 2 other patients showed a normal triglyceride content. Only 2 patients in the coronary group showed normal clearing at 24 hours.

Vitamin A Content of D < 1.006 and D > 1.006 Particles

At 4 hours slightly more vitamin A (V-A) was found in D < 1.006 than in D > 1.006 particles. The V A content of both particles was higher on the

ABLE 14. Vitamin A content (I.U./100 ml) of D < 1.006 and D > 1.006 particles in sera of healthy rsons and survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate d 100 ml of cream (40 per cent fat) Data corrected to 100 per cent recovery of total plasma content.

Subjects	D < 1.006											
	4 hours			6 hours			10 hours			24 hours		
	N	Mean	S.D	N	Mean	S.D	N	Mean	S.D	N	Mean	S.D
ealthy	9	1383	805	8	1207	748	8	749	461	9	60	43
oronary	12	1725	1032	12	3014	1417	10	2794	2412	11	287	279

Subjects	D. > 1.006											
	4 hours			6 hours			10 hours			24 hours		
	N	Mean	S.D	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D
lealthy	9	1029	536	8	1565	700	8	1127	351	9	439	480
oronary	12	1212	915	12	2129	691	10	2706	1979	11	871	846

average, in coronary patients than in healthy subjects, but the distribution pattern was equal in both groups. Thus, contrary to the findings in the whole V A loading series, presented above this small coronary group showed already at 4 hours a higher mean total content of V A than the healthy group.

The difference in the average total plasma vitamin A in the healthy and the coronary groups (calculated from table 14) became more apparent at the time interval from *4 to 6 hours*. This was chiefly due to vitamin transported in the light particles (D < 1.006) which showed a sharp increase up to 6 hours in the coronary group, while in healthy persons the peak level was reached already at 4 hours. V A in the D > 1.006 particles showed a definite increase from 4 to 6 hours in both groups, the rise being more marked in the coronary group. Accordingly as seen in fig. 28, the percentile distribution of vitamin A showed a considerable change towards the D > 1.006 particles in the healthy group.

The mean peak level of plasma total vitamin A occurred in the healthy group *at 6 hours* and in the coronary group 4 hours later. This delayed peaking is reflected nicely in the lipoprotein concentration curves (fig. 27). Thus, V A in fraction D > 1.006 in the coronary group showed on an average a marked increase up to 10 hours, while in healthy persons V A in this fraction was then already continuously decreasing after the peak level had been attained at 6 hours. The vitamin in the light particles showed a decrease

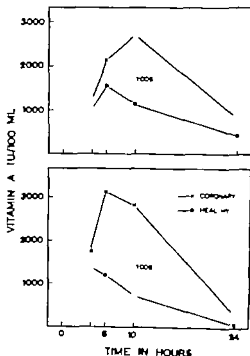


Fig. 27 Vitamin A content of D < 1.006 and D. > 1.006 particles in 9 healthy persons and 12 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat)

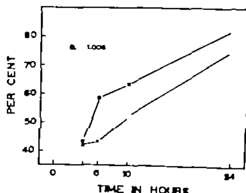


Fig. 28. Vitamin A content of D. > 1.006 particles as per cent of total plasma content in 9 healthy persons and 12 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat)
— healthy x — coronary

present in the high density lipoproteins. In the coronary group, on the other hand the contents in D 1 006—1.019 and D 1.019—1 063 at 24 hours were almost identical and greater than in the high density lipoproteins.

The percentile distribution curves of the D 1.006—1.019 particles had a parallel course in both groups. A «crossing-over» occurred in the per centile distribution curves of D 1.019 —1.063, showing a dominance of this fraction at a later phase in the coronary group as compared to the healthy group. Simultaneously with this, the percentile distribution curves of the D > 1.063 fraction diverged, indicating a higher proportion of this fraction in the healthy group

These data are in many respects inadequate and do not have the weight of statistically corroborated evidence Thus it is not possible on the basis of these few observations to demonstrate the kinetics of vitamin A lipoprotein, nor the presence of a possible defect in this respect in coronary heart disease However some tentative conclusions may be justified. These data suggest that the newly absorbed vitamin A is carried primarily in the particles D < 1.006 and D 1 006—1.019 The major difference between the coronary and control groups existed initially in the two fractions of lowest density (D < 1.006 and D 1 006—1 019) In the later phase however the kinetics of vitamin A in the more dense fractions were dissimilar The disappearance rate from particles D 1 019—1 063 was slower in the coronary group and the percentile increase of vitamin A in the D > 1.063 fraction was higher in the healthy group. At 24 hour the latter fraction contained most of the vitamin in the healthy group.

DISCUSSION

SERUM BASAL LIPID LEVELS

There is probably good reason to assume that the blood donors represent in regard to the plasma lipid pattern a fairly unselected Finnish ur ban population. The samples for the cholesterol and tocopherol determinations of these subjects were taken in the non-fasting state. The plasma tocopherol level, however is affected neither by an ordinary meal nor by a fat load, as was demonstrated in the present study and by other investigators (McCormick *et al.* 1960) Only a slight elevation of the serum total cholesterol has been observed after a fat meal (Havel 1957) No significant difference was found in the serum total cho.-esterol and tocopherol levels of blood donors and healthy subjects of the same age studied in the fasting state.

All the coronary patients had electrocardiographic evidence of a recent and/or old myocardial infarction. Most of the blood samples of coronary patients were taken in the fifth week after the acute attack of myocardial infarction or angina pectoris. Therefore it cannot be concluded whether or not the high plasma lipid level found in the coronary patients existed also prior t the acute event. However if the changes in the serum cholesterol values occurring at the acute stage of myocardial infarction are omitted, no essential differences in this respect have been found before and after the event (Gordon *et al.* 1959 Page and Lewis 1959)

The serum cholesterol level has been observed to decrease at the acute stage of myocardial infarction by many authors (Welin 1948 Björck *et al.* 1957 Dodds and Mills 1957 Pomerantz 1962) and the present results confirm this. A similar trend has been reported in the serum triglyceride level (Hauss and Böhle 1955 Albrink *et al* 1961) In the present investigation, however no significant fall was found in the serum triglyceride level.

A decrease of serum lipids has also been observed after a surgical opera tion (Man *et al.* 1946) In many respects the postinfarctive and postoper ative states are analogous, with tissue destruction fever and leukocytosis. The similar behavior of the serum cholesterol and tocopherol levels observed in the present study — the latter being purely an exogenous lipid — makes a defect in the lipid synthesis unlikely as a cause of the fall of the

serum lipid. The changes are probably too acute to be due to a poor intake, and thus the best explanation seems to be the increased catabolism or excretion of these lipids, as has been suggested by Pomerantz (1962)

The present coronary population showed, on the average a significantly higher serum content of all the four lipids — total cholesterol, triglyceride tocopherol and vitamin A — than the control population. The elevation of serum cholesterol, triglyceride and tocopherol in the coronary series was more marked in the younger persons and there appeared to be an almost continuous decrease in the plasma level of these three lipids with increasing age of the population from the 4th to the 7th decade However when the coronary series was divided into two age groups aged below and over 50 years, no significant difference was found in the lipid levels in these groups. This may be explained by a marked decline in the lipid values after the age of 40 years. The vitamin A series was too small to permit a study of the possible age trend, but also here the two age groups showed similar mean vitamin A values.

The control subjects, on the other hand, showed an opposite age trend. A continuous increase of the serum cholesterol and tocopherol levels occurred up to the 7th decade while the triglyceride and vitamin A series were too small to allow such treatment. When the control groups were divided into two age groups aged below and over 35 years, the younger group had a significantly lower mean content of all the lipids with the exception of vitamin A. Thus, with respect to the serum cholesterol and tocopherol values, which were analyzed from a sufficient number of samples, no difference between the coronary and control groups existed any longer in the 7th decade

The influence of aging on the plasma lipid or lipoprotein levels is a matter of great importance. Evidently there exists a higher death rate among subjects with high lipid levels, which partly explains the diminution of the serum cholesterol level with aging in the coronary population. However as Lawry *et al.* (1957) emphasized, there must be another more important mechanism responsible for this phenomenon. According to Albrink *et al.* (1961) a marked rise in the serum triglyceride level with aging occurs in healthy men in the thirties or fourties, preceding by about twenty years the peak incidence of coronary heart disease and thus supporting the hypothesis that a serum lipid elevation is the first detectable evidence of the slowly developing chronic process. Supporting this view it has been shown that the increase of serum cholesterol with aging is not an obligatory one but that the cholesterol level increases only in some persons (Sperry and Webb 1950 Man and Peters 1953) The young coronary patients, who show a particularly high incidence of hyperlipidemias, may probably derive from a somewhat different population than the aged coronary patients (Björck *et al.* 1957) Among the old patients, on the other hand the high lipid levels may

partly be due to the physiological rise of serum lipids with age as is the case concerning the cholesterol content of the aorta. It has been shown by Faber (1946) that the cholesterol content of autopsied aortas increased with age in normocholesterolemic subjects but not in hypercholesterolemic subjects.

Serum Cholesterol Level. — The present observation of a higher mean serum total cholesterol level in persons who had had a myocardial infarction as compared to healthy persons of comparable age has also been reported by numerous authors, as was stated in the review of the literature. In longitudinal studies, also the persons who developed coronary heart disease during the time of observation have been reported to exhibit a higher serum cholesterol level than the base population (Gofman *et al.* 1956, Kannel *et al.* 1961)

The increase in the serum cholesterol level with aging up to the 7th decade in the normal population found in this study has been observed in population studies by numerous investigators (Gertler *et al.* 1950 b, Keys *et al.* 1950, Jones *et al.* 1951, Nikkilä 1955, Lawry *et al.* 1957 Lewis *et al.* 1957) but not by all (Little *et al.* 1956 Oliver and Boyd 1956)

Serum Triglyceride Level. — The incidence of hypertriglyceridemia in the coronary population in the present study was somewhat higher than that of the other hyperlipidemias. This is in accord with the data in the literature (Hauss and Böhle 1955, Schrade *et al.* 1959 1960 1961 Antonis and Bersohn 1960 Albrink *et al.* 1961, Berkowitz and Croll 1962) On the other hand in a recent paper by Nikkilä and Pelkonen (1963) the incidence of hypertriglyceridemia in a somewhat different Finnish coronary population was lower than in the present study However a large proportion of that coronary population showed serum triglyceride values at the upper limit of the normal range (120—140 mg/100 ml) the normal maximum being 140 mg/100 ml.

The present data concerning the influence of age on the serum triglyceride level in the normal population is also in agreement with earlier reports (Hauss and Böhle 1955, Schrade *et al.* 1960 Antonis and Bersohn 1960, Carlson 1960 a, Albrink *et al.* 1961, Cramér 1962) In the coronary population, on the other hand Carlson (1960 b) stated that the metabolism of triglyceride was more often disturbed in men below the age of 50 years than after this age while the situation was the opposite in the study of Albrink *et al.* (1961) In the present study the highest mean level was observed at 30 to 39 years of age, but the mean levels in the age groups below and over 50 years were similar Accordingly the incidence of hypertriglyceridemia was almost equally high in the two age groups, being 70 per cent in the younger and 65 in the older group, if the normal limits are defined according to the distribution of values in the young population.

Plasma Tocopherol Level. — In the present study the mean plasma tocopherol level in the blood donors, repre-

senting broadly a Finnish normal urban population appeared to be lower than the earlier reported data from Holland (Engel 1949) Hungary (Kramer 1955) Italy (Rindi and Perry 1957) England (Leitner *et al.* 1960 b) and the United States (Harris *et al.* 1961) but of the same order of magnitude as that in the report of Postel (1956) also from the United States. The only data reported from Finland, 5.1 mg/L for women and 4.2 mg/L for men (Rauramo 1946) are considerably lower than the present averages.

The present observations concerning the age dependence of the plasma tocopherol content in the normal population and in women and men separately are in good accordance with the data reported earlier (Darby *et al.* 1949 Lemley *et al.* 1949 Chiaffi and Kirk 1951, Leitner *et al.* 1960 b)

There is no data available on the plasma level of tocopherol in coronary heart disease However Vannotti and Gervasoni (1957) and McCormick and McCluer (1960) observed that tocopherol was present in human atheromas. The latter finding is interesting in the light of the present study which definitely revealed a significantly higher mean plasma tocopherol level in patients with myocardial infarction than in control subjects. The presence of a high content of tocopherol in atheromas and in plasma in atherosclerosis is thus analogous to that of cholesterol, triglyceride and vitamin A

The serum cholesterol and tocopherol levels appeared to be in close correlation of a high statistical significance. The possibility of a methodological error was excluded in this study as it had been in the study of Postel (1956) A positive correlation between the serum cholesterol and tocopherol levels has been reported in diabetic patients (Bensley *et al.* 1950 Vanzetti *et al.* 1956) and in thyroid disorders (Postel 1956) In addition, high serum tocopherol levels have been observed to exist in hypercholesterolemic states (Darby *et al.* 1949) Darby and co-workers postulated that the simultaneous occurrence of hypercholesterolemia and hypertocopherolemia was due to an increased lipid carrying power of serum This means, in modern terminology hyperlipoproteinemia, which, indeed evidently exists in coronary heart disease Furthermore the distribution of cholesterol and tocopherol in the plasma lipoproteins is very similar (Bragdon *et al.* 1956 McCormick *et al.* 1960) Postel also considered it unlikely that the changes in the serum tocopherol content observed in thyroid disorders were determined by the serum cholesterol content. In his opinion the thyroid activity appears to dictate the rate of synthesis, degradation and excretion of cholesterol, while the regulation of serum tocopherol is determined primarily by the intake, absorption and rate of disposal without the component of synthesis. A common excretion pathway as well as a similar enterohepatic circulation of cholesterol and tocopherol have also been suggested by some authors (Klatskin and Molander 1952 a, Popper *et al.* 1949 Simon *et al.* 1956 a) With the exception of the endogenic synthesis, the metabolism of serum

tocopherol and cholesterol thus have so many similarities that the correlation between the serum levels does not seem to be unexpected.

A highly significant correlation between the serum triglyceride and tocopherol levels was found in the coronary group but not in the healthy group. According to the known lipid composition of lipoprotein, most of the triglyceride appears to be in particles D < 1.019 (Bragdon *et al.* 1956) On the other hand, in the study of McCormick *et al.* (1960) the major part of the plasma tocopherol was in the Sf 3—9 particles and in the present study in the D 1.019—1.063 particles. In coronary heart disease, the lighter beta-lipoproteins Sf 12—400 have been found to be elevated more than the Sf 0—12 lipoproteins (Gofman *et al.* 1954) According to the present observations, relatively more tocopherol was present in D 1.006—1.019 particles in the coronary patients than in healthy persons. The significant correlation between the serum triglyceride and tocopherol levels in the coronary group may thus partly be due to a shift of tocopherol in the lipoprotein spectrum toward lighter particles, in analogy to the shift of cholesterol in the lipoprotein spectrum in hypertriglyceridemias (Albrink 1961)

Plasma Vitamin A Level. — The mean vitamin A level in healthy persons in the present study was lower than the earlier reported mean levels, this concerning also the data published from Finland (Saksela 1940 Pitkänen 1944) The data on the influence of age on the plasma vitamin A level are controversial. In the studies of Saksela (1940) and Vetter (1953) the plasma vitamin A level was not age-dependent, while Leitner *et al.* (1960 a) reported an increase with age. In the present study the older healthy individuals (age over 35 years) had a higher mean level than the younger ones, but the difference was not significant.

Carotenoids have been found to be present in human atheromas (Thomson 1934, Blankenhorn *et al.* 1956) A higher mean plasma vitamin A level has been seen in coronary patients than in control ones, supporting the present observation (Beaumont *et al.* 1958, Beaumont and Lenègre 1959) In various hyperlipidemic states hypercarotenemia and hypervitaminosis A have been frequently observed, as was shown in the review of the literature Many theories have been presented to explain this phenomenon. A defect in the conversion of beta-carotene to vitamin A has been postulated as the reason for the hypercarotenemia (Halli *et al.* 1936, Cohen 1958) but the evidence is poor at least in diabetes (Kimble *et al.* 1946) In the case of hypervitaminosis A a pathologic affinity to serum» or an increased solubility in the serum were suggested in the early studies (Lindqvist 1938, Popper *et al.* 1948) These suggestions alluded thus to an increased lipid or lipoprotein level in plasma, to an abnormal vitamin A-lipoprotein linkage, to an increased influx into or a defect in the removal of vitamin A from circulation. The latter has been nicely shown by Kagan *et al.* (1950) in nephrotic child

ren in a case of hyperlipemia associated with coronary heart disease by Martt and Connor (1956) and in patients with angina pectoris by the French investigator group (Beaumont *et al.* 1958)

In the present study the younger control group (age below 35 years) was used as the normal population for comparison because of the rather high incidence of clinically undetected atherosclerotic lesions at a later age (Dock 1959) The plasma content that was exceeded only by 10 per cent of the younger control subjects was defined as the normal value

On the basis of these criteria of normality hypertriglyceridemia appeared to be the most common lipid abnormality of plasma. In 44 per cent of the coronary group all the lipids (cholesterol, triglyceride tocopherol) were simultaneously abnormally high and 17 per cent of the cases showed a completely normal serum lipid pattern. On the other hand, 27 per cent of the patients has only one abnormally high serum lipid level.

Two basic considerations seem to be at hand on the basis of the present lipid measurements. Firstly there exists in the coronary population no typical serum lipid pattern or single metabolic defect affecting only one serum lipid, but a universal tendency to the occurrence of hyperlipidemias. Secondly without regard to the quality of the lipids, young coronary patients show a slightly higher frequency of hyperlipidemia than those of more advanced age

KINETIC ASPECTS OF PLASMA LIPIDS AND THEIR TRANSPORT

A prolonged lipemia is frequently found in patients with coronary heart disease. However the disappearance rate of lipids administered intravenously seems to be normal in coronary heart disease. This apparent discrepancy may probably be explained by the recently expressed view that artifical fat emulsions and alimentary chylomicra are removed from the blood circulation in a different manner The reticulo-endothelial system seems to be more important in phagocytizing the artificial oil emulsions, while the chylomicra of alimentary origin are removed by the parenchymal cells of the liver (DiLuzio 1960 Dole and Hamlin 1962)

In the present study the great majority of the members of the coronary group showed a more marked and prolonged tocopherolemia than the control group after oral administration of the vitamin. A similar abnormality was found in the vitamin A studies. Furthermore the peak level of plasma vitamin A tended to occur later in the coronary patients than in the healthy subjects. Comparison of the results obtained in the loading tests and in the serum fasting lipid measurements revealed further that the metabolic

abnormality was best disclosed by the loading tests. Of some importance is probably the observation that the plasma tocopherol levels at 24 hours were significantly correlated to the fasting serum cholesterol, triglyceride and tocopherol levels. This may suggest that a slow clearance rate of lipids is one of the important factors in the genesis of hyperlipidemias.

The plasma tocopherol and vitamin A are both carried completely by the lipoproteins, but in a somewhat different manner. The lightest particles D < 1.006 seemed to be quantitatively less important in the tocopherol transport than in the vitamin A binding. On the other hand, the more long lived D 1.019—1.063 particles accounted for the major portion of the plasma tocopherol, while only 10—25 per cent of vitamin A was found in these particles. However since no actual turnover studies were done, the possibility cannot be excluded that the relatively low tocopherol content of the short-lived D < 1.006 particles may in fact reflect only a higher turnover rate of tocopherol in these particles.

In spite of the absence of an endogenous synthesis of these two vitamins, there are still too many unknown factors that preclude an accurate interpretation of lipoprotein vitamin kinetics on the basis of the results obtained. However some more or less speculative considerations may be justified.

It seems unlikely that an increased absorption from the intestine is the major cause of the abnormal lipemia in coronary subjects. The difference in the plasma lipid content in healthy subjects and coronary patients tends to become the more apparent the longer the time since the ingestion of the fat. On the other hand, a deficient lipolysis in the gastrointestinal tract has been postulated by Marks *et al.* (1962) as the reason for the high postprandial lipemia. A low blood lipase activity has also been reported in association with high postprandial lipemias (Tietz *et al.* 1960)

In discussing the causes of hyperlipidemia, most authors agree that one of the defects is in the removal of lipids from the circulation. Undoubtedly the lipoprotein lipase liberated into the circulation after the administration of heparin is capable of clearing the lipemic postprandial plasma. However the role of endogenous lipoprotein lipase in the normal lipid metabolism is still a matter of controversial opinion (Engelberg 1960 Olson and Vester 1960 Dole and Hamlin 1962) The viewpoint that endogenous lipoprotein lipase has a minor role has originated from the low amount present in the plasma (Gates and Gordon 1958) Studies of the clearing effect of heparin on the postprandial lipemia in coronary heart disease have led to contradictory results (Block *et al.* 1951, Mitchell and Bronte-Stewart 1959) That hyperchylomicronemia actually follows a deficiency of this enzyme has been demonstrated by Havel and Gordon (1960) in describing a family with a lipoprotein lipase defect.

The injection of heparin has been shown to decrease the total plasma vitamin A content, with a concomitant increase of the free vitamin A alcohol

(Schrieck and Kunkel 1956) In the study of Beaumont and Beaumont (1961) the response to vitamin A load diminished also after the administration of heparin to 3 hyperlipidemic patients. They observed in addition a decrease in the vitamin A content of the chylomicra, while the vitamin content of the beta-lipoproteins separated by dextran sulphate increased. Therefore, theoretically a defect in an enzyme system sensitive to heparin, analogous to lipoprotein lipase may be responsible for the abnormal vitamin A metabolism in coronary heart disease and in hyper lipidemia. In the light of this considera tion the observation of Popper *et al.* (1948) that the elevated plasma vita min A level in nephrotic sera was chiefly due to the vitamin A ester is of interest. In this connection it is also wortwhile to note that in the present study most of the plasma vitamin A at 24 hours was in the D > 1.063 particles in healthy persons, but that in coronary patients this fraction contained the least vitamin A. The D > 1.063 particles have been found to be the chief carriers of the free vitamin A alcohol (Krinsky *et al.* 1958) The increase in vitamin A alcohol observed after the injection of heparin occurred in the alpha lipoprotein fraction (Schrieck and Kunkel 1956) that corresponds to the D > 1.063 fraction in the present study A defect in the vitamin A »clearing facto (esterase) may thus result in a low itamin A content in high density lipoproteins.

According to Nikkilä and Pelkonen (1962 a) the administration of heparin did not influence the plasma tocopherol level during the tocopherol loading test. The reason for the insensitivity of tocopherol to heparin may lie in the fact that, contrary to vitamin A, tocopherol is absorbed as free alcohol (Week *et al* 1952)

The role of the various organs in the removal of alimentary chylomicra is not adequately known. However the liver evidently is one of the central organs in this process. It has been shown that the newly absorbed vita min A esters are phagocytized directly by Kupffer s cells of the liver while the free vitamin A alcohol is deposited after hydrolysis into the parenchymal cells of the liver (Glover and Morton 1948) The data available concerning tocopherol removal are few but in studies with labeled tocopherol the maximum radioactivity in the liver was obtained one hour after ingestion of the label and a second peak occurred at 32 hours (Sternberg and Pascoe-Dawson 1959) A recent study by DiLuzio (1960) indicated that the reticulo-endothelial system has an essential role in the cholesterol metabolism. He was able to obtain a profound lowering of the serum cholesterol level in rats after inducing hyperfunction of the reticulo-endothelial system. On the other hand, blockage of the reticulo-endothelial system has been found to manifest in an increase of the plasma cholesterol and vitamin A levels and in a slowing down of the disappearance rate of vitamin A in experimental animals (Brown *et al.* 1952) A common metabolic pathway of serum cholesterol and vitamin A may also be sug-

gested on the basis of the present results, according to which there was a significant correlation of the serum cholesterol and 24-hour vitamin A levels. An insufficient function of the reticulo-endothelial system should thus probably be considered to be one of the possible mechanisms in the development of hyperlipidemia.

The half life of circulating plasma lipoproteins has been shown to increase with increasing density (Fredrickson *et al.* 1958, Gitlin *et al.* 1958, Edgren 1960) Conversion of Sf 10—100 lipoproteins to Sf 3—9 lipoproteins has been observed (Gitlin *et al.* 1958) On the other hand, a similar protein has been found to be present both in the chylomicron fraction and in the high density lipoproteins (D 1.063—1.21) which therefore have been thought to comprise one metabolic unit (Rodbell *et al.* 1959)

The present studies of tocopherol kinetics showed in coronary heart disease a high percentage of tocopherol in the D 1.006—1.019 particles and a significantly decreased disappearance rate of tocopherol from the D 1.019—1.063 particles. A defect in the conversion of Sf 10—100 lipoproteins to Sf 3—9 lipoproteins has been presented by Gitlin *et al.* (1958) in nephrosis. It has also been emphasized by George *et al.* (1961) that a slow disappearance rate of labeled neutral fats in coronary heart disease is more compatible with a defect in the metabolism of the Sf 20—400 lipoproteins than in that of the chylomicra. Tocopherol is insensitive to heparin and thus the present results support the finding of George *et al.* and Gitlin *et al.* showing a retention of tocopherol in the D 1.006—1.019 particles.

In this connection it is noteworthy that according to the observation of Hanig *et al.* (1956) the Sf 12—100 lipoprotein is always present in substantial amounts in aortas where there is atherosclerotic activity while the Sf 0—12 lipoprotein is absent almost completely in the aorta extracts.

Vitamin A studies showed in coronary patients an accumulation of the vitamin in the D < 1.006 and D 1.006—1.019 particles. The consideration presented above is compatible also with the abnormal vitamin A kinetics in the D 1.006—1.019 particles. On the other hand, a high content of vitamin A in the D < 1 006 particles and a low content of the vitamin in the D > 1.063 particles possibly suggest a defect in the heparin-sensitive system.

Hyperlipidemia may theoretically be the result of an overproduction of the plasma lipids or of a defect in the removal of the lipids from the circulation. The present studies with purely exogenous lipids demonstrate that the lipid removal, at least, may be impaired. The present results support the view that the defect in fat disposal may lie in various steps of the complex process, which is in a good accordance with the heterogeneity of the hyperlipidemias found by measurement of the fasting plasma lipid values in coronary heart disease.

SUMMARY

The object of the present investigation was to study the metabolism of vitamins A and E, representing purely exogenous lipids, in coronary heart disease In order to classify the lipid disorder of the subjects the serum total cholesterol and triglyceride levels were also determined.

The control subjects were 176 male and 146 female blood donors and 88 male and 13 female healthy subjects. The blood samples from blood donors were taken in the non fasting condi tions. The blood donors underwent no medical examination. The healthy subjects were either medical students, all subjectively healthy or patients under medical observation in the hospital. The patients included in the healthy group were admitted to the hospital for a minor congenital heart disease or the medical examination revealed no organic disorders.

The coronary group was comprised of 110 male and 14 female survivors of myocardial infarction. All the patients were admitted to the hospital because of an acute attack of myocardial infarction or of angina pectoris and all had electrocardiographic evidence of an old or recent myocardial infarction. The blood samples were not taken before at least 3 weeks had elapsed from the acute attack, and most of the samples were taken 4 weeks after ad mission.

Serum Total Cholesterol Level. — The blood donors showed a continuous increase of the mean cholesterol levels from the 3th to 7th decade. In the coronary group, on the other side definite decreases of serum cholesterol levels occurred from the 4th to 5th and from the 6th to 7th decade The mean levels of coronary patients below and over 50 years did not, however differ significantly

The coronary group showed a significantly higher mean level than the blood donors. The difference was also significant when the age groups of 30—39 40—49 and 50—59 years in the two populations were compared. Aged per sons in the 7th decade however sowed no difference.

Serum Triglyceride Level. — The older healthy subjects over age 35 showed a significantly higher mean level than the younger subjects. In the coronary group there was a tendency to decreased levels with increasing age However no significant difference was found in the mean values in the two age groups (age below and over 50 years)

The coronary group showed a signi

ficantly higher mean level than the healthy subjects. The difference was also significant when the age groups 30—39 and 50—59 years of the two populations were compared.

Plasma Tocopherol Level. — The blood donors showed an almost continuous increase of the mean levels from the 3th to the 7th decade An opposite age trend was seen in the coronary population.

The coronary group had a signifi cantly higher mean level than the blood donors. The difference was also significant in the age groups from the 4th to 6th decade, but not in the 7th decade.

Plasma Vitamin A Level. — In the healthy population no significant dif ference existed between the mean levels in the two age groups (age over and below 35 years) The two coronary age groups (age below and over 50 years) showed also similar mean levels. The coronary group had a significantly higher mean level than the control group.

Incidence of Hyperlipidemia in Sur vivors of Myocardial Infarction. — The plasma content that was exceeded only by 10 per cent of the younger control subjects (age below 35 years) was used as the normal value. The normal values of the serum total cholesterol, triglyceride tocopherol and vitamin A contents were 290 mg/100 ml, 125 mg/ 100 ml, 15 mg/L, and 156 I.U./100 ml, respectively

The incidence of hypercholesterol emia in the whole coronary population was 60 per cent, being 70 per cent for the younger (age below 50 years) and 50 per cent for the older patients. The corresponding percentages of hypertriglyceridemia were 68, 70 and 65 per cent those of hypertocopherolemia 53 55 and 50 per cent, and those of hyper vitaminosis A 59 64 and 55 per cent, respectively

Interrelationships of the Serum Lipid Levels. — The regression analyses revealed a significant regression between the serum cholesterol and tocopherol levels in the coronary group and in the combined control group consisting of the blood donors and the healthy subjects. The serum triglycer ide and tocopherol levels were significantly correlated in the coronary group only The serum triglyceride and cholesterol levels also showed a significant correlation in both groups, the correlation being closer in the coronary group.

The plasma vitamin A level did not correlate significantly with the serum cholesterol or triglyceride levels.

The lipid levels were followed in 9 patients after the acute event until at least 5 months had elapsed. A signifi cant fall in the serum cholesterol and tocopherol levels was observed during the first week. After at least 5 months had elapsed from the acute attack the initial values were reached. The variations observed in the serum triglycer ide levels were not significant.

Tocopherol Loading Test. — To 38 healthy subjects and 64 survivors of myocardial infarction 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat) were given, after which blood samples for tocopherol

determination at 4, 6, 10 and 24 hours were taken.

The healthy subjects showed a higher initial increase than the coronary subjects. However a significantly higher concentration curve was found in the coronary group than in the healthy group. The difference was as signifi cant when the older healthy and the younger coronary groups of similar mean ages were compared. The 24-hour tocopherel level was found to have a significant correlation to the plasma basal tocopherol, cholesterol and triglyceride values in both groups. The plasma 24-hour tocopherol content of 24.5 mg/L was exceded by 10 per cent of the younger healthy subjects, by 33 per cent of the older healthy persons, and by 83 per cent of the coronary group.

Vitamin A Loading Test. — To 36 healthy subjects and 34 survivors of myocardial infarction 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat) were given. In the complete test the blood samples for vitamin A determination were taken 2, 3, 4 6 10 24 hours after administration of the vitamin.

The peak level occurred from 4 to 10 hours after administration of the vita min and was significantly higher in the coronary group than in healthy per sons. Furthermore the peak levels tended to occur later in the coronary than in the healthy group. The healthy persons showed a more rapid initial increase Later however the mean increase from the basal level as well as the mean levels were significantly higher in the coronary group than in the healthy group.

None of the subjects had reached the initial plasma content at 24 hours. A significant correlation between the plasma basal and 24 hour vitamin A levels was present in the healthy group. The basal cholesterol and the 24-hour vitamin A levels were in a significant correlation in both the groups.

The plasma 24-hour vitamin A con tent of 450 I.U./100 ml was exceded only by 10 per cent of the younger healthy subjects, by 2 of 10 older healthy subjects only 3 of the 29 patients in the coronary group were below this limit.

In order to study the *Vitamin A and E kinetics* both of these were analyzed from 5 lipoprotein fractions. The plasma lipoproteins were isolated in a Spinco Model L preparative ultracentrifuge by two successive runs. In the primary separation D < 1.006 and D > 1 006 fractions were obtained. In the secondary separation the D > 1.006 fraction was separated further and D 1.006—1.019 D 1.019—1.063 and D > 1.063 fractions were obtained.

The primary separation for further determination of the tocopherol con tent was performed from fasting plasma and from samples taken from 25 healthy subjects and 17 survivors of myocardial infarction 4 6, 10 and 24 hours after administration of the tocopherol. The secondary separation was performed from fasting plasma and from samples taken from 7 healthy subjects and 8 survivors of myocardial infarction 4, 10 and 24 hours after the tocopherol load.

In vitamin A studies the primary separation of plasma lipoproteins was performed from blood samples taken from 9 healthy subjects and 12 survivors of myocardial infarction 4, 6, 10 and 24 hours after the administration of vitamin A. The fraction D > 1.006 was separated further of the plasma of all the healthy subjects and 9 survivors of myocardial infarction

Tocopherol in Plasma Lipoproteins. — Tocopherol was found to be present in all the fractions isolated, most of it being in the D 1.019—1.063 particles. The most characteristic finding in the coronary group was the high percentage of tocopherol in the D 1.006—1.019 particles.

The newly absorbed tocopherol was carried primarily in the D < 1.006 and D 1.006—1.019 particles; later however the major part of the newly absorbed tocopherol was found in the D 1.019—1.063 particles. The D > 1.063 particles were quantitatively less important. The coronary group showed a marked retention of tocopherol in the D 1.006—1.019 particles and a significantly slow disappearance of tocopherol from the D 1.019—1.063 particles.

Vitamin A in Plasma Lipoproteins — The newly absorbed vitamin A appeared to be carried primarily in the D <1.006 and D 1.006—1.019 particles. At 24 hours in the healthy group, however most of the vitamin A was found in the D > 1.063 particles. The major abnormality in the coronary group was the high vitamin A content in the D < 1.006 and D 1006—1.019 particles. The disappearance rate of vitamin A from the D 1.019—1.063 particles was significantly slow and the percentile increase of vitamin A in the D > 1.063 particles was also low in the coronary group.

The error of lipid metabolism in coronary heart disease was discussed. The possible role of the endogenous clearing factor and of the reticulo-endothelial system in the pathogenesis of hyperlipidemias was emphasized.

It was concluded that the disappearance rate of exogenous lipids from the blood circulation may be impaired in patients with coronary heart disease and that there exists no single defect in the lipid metabolism but a universal tendency to hyperlipidemia.

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ACUTE FLUORIDE INTOXICATION

By

G. L. Waldbott

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CONTENTS

INTRODUCTION

The element fluorine, a pale yellow gas, condenses to liquid at 127 C, freezes at —250° C. It is the most reactive of all elements (1) Because of its strong tendency to combine with other elements, fluorine rarely occurs in pure gaseous form.

The ion fluoride (F) as a part of F compounds, is very widespread in nature. It is estimated as the 13th in abundance among the elements of the earth (2) (3) Pharmacologically significant is its strong affinity to Ca++ and other metals with which it can enter into highly complex compounds.

Formerly accidental exposure to F was occasioned mainly through its extensive use as an insecticide and rodent exterminator A by-product of many industrial processes, it early received attention as an occupational hazard. In recent decades F is playing an ever increasing role in industry

Among the numerous processes in which its compounds have been employed *since the beginning of the 20th Century* are manufacturing of aluminum, superphosphate fertilizer steel, magnesium, enamel, pottery glass, bricks, beryllium, zirconium, tantalum and niobium. They are also used in welding, in the cleaning industry and as a preservative.

During the 1930's F compounds began to enter into the refrigerant, aerosol, lubricant and plastic fields.

During the last two decades their use expanded into separation of uranium isotopes by gaseous diffusion. F compounds are now used extensively as propellants in our missile program and in the manufacture of steroids, tranquilizers, diuretics, antimetabolites, anticancer drugs, antihistamines, anesthetics, androgens estrogens. Fluoride liberated from burning coal and oil, emanates from smokestacks of industrial plants causing air contamination which has contributed to major disasters (5, 6 7 8)

Less significant as a source of acute intoxication is its presence in food and water It has been demonstrated that F in water (9) may lead to acute allergic reactions.

Through all these media, F is liable to be taken into the system.

A. F Toxicity

The toxicity of F is based on its strong reactivity which exceeds that of chlorine and oxygen.

Toxicity of F compounds depends largely on their solubility and the degree of dissociation of the F ion.

The more soluble compounds such as sodium fluoride, sodium silicofluoride (Na_2SiF_6) or hydrogen fluoride (HF) are more toxic than the less soluble ones, e.g cryolite (Na_3AlF_6) and calcium fluoride (CaF)

The stronger the bond between the F and other ions, the more inert the compound. Potassium fluoroborate (10) or potassium hexafluorophosphate (11) pass through the body without releasing F ions. Its close linkage to the molecule renders the F compound inert. HF is the only compound which penetrates the body as an un-ionized acid but thereafter it is diluted and ionized to give F ions.

Table I Comparative lethal dose of fluoroacetate in various animals (compiled from data presented by Bredemann (12)

	mg/kg
Dog	0.06
Rabbit	0.2
Cat	0.5
Swine	0.3
Sheep (15)	0.4
Goat	0.7
Horse	1.0
Sparrow	2.7
Rat	5.0
Rhesus monkey	5.0–7.5
Chicken	6.0–7.0
Pigeon	10.0
Monkey	15.0
Frog	500.0
Humans	1–5

In most inorganic fluorides, the F ion determines their toxicological behavior (12). In contrast, the F ion in organic compounds usually contributes less to the toxicity. Therefore data on toxicity are concerned mainly with inorganic fluorides.

1 ORGANIC COMPOUNDS

The number of organic fluorides i.e. those with F bound to carbon is legion. Only those significant in medical practice will be discussed here.

Organic F compounds are not likely to occur in food. In tea, fish and gelatin, which contain high F levels, it is believed to be present in inorganic salts.

Organic fluorides can be grouped toxicologically into 3 main categories: Fluoroacetates, Fluorophosphates and Fluorocarbons.

Sodium fluoroacetate the commercial rodenticide 1080 deserves special attention because of its toxicity to humans and to animals. It is a powder with a faint vinegar like odor. As little as 0.1 mg/kg has proved fatal in dogs and cats (13). In humans the mean lethal dose ranges from 1 to 5 mg/kg.

Biologically fluoroacetate is a "delayed convulsant". Initial symptoms do not occur for several hours. When fluoroacetate reaches the system, it is converted into fluorocitrate (14). This process requires time. Fluorocitrate blocks the tricarboxylic acid cycle, an essential mechanism of energy production. This is followed by glycogen depletion in liver, hyperglycemia, increase in blood lactate, ventricular fibrillation, tetaniform convulsions. It leads to respiratory paralysis (15). Citrate levels in blood, urine and kidneys rise, urinary calcium excretion decreases (16). The citrate levels in kidneys appear to regulate urinary calcium excretion.

Fluoroacetates occur in nature in the leaves of a plant growing in South Africa Dichapetalum cymosum (called Gifblaar). Cattle and sheep grazing on this plant are known to have died. Di and tri fluoracetic acid are much less toxic than monofluoracetic acid (12).

In another plant Dichapetalum toxicarium (16 a) the seed contains a certain fluorofatty acid after ingestion of which death in sheep may be delayed up to 5 days, a fact which is believed to explain some casualties of unknown cause in Africa.

Table I illustrates the comparative toxicity of 1,080 in various animals.

The fluorophosphates[1] include diisopropylfluorophosphate (DFP) and Sarin, both extremely toxic gases used in chemical warfare. Even as minute a dose as 0.05 mg

[1] Fluorophosphates are usually grouped with organic fluorides although they contain no carbon.

applied to the eye of a guinea pig produces death within a few minutes (17) The toxic effect of fluorophosphate is due to inhibition of cholinesterase, an enzyme vital in the process of nerve transmission. Concentrations as low as 10^{-8} produce this effect. Inactivation of serum cholinesterase by DFP is irreversible.

Most fluorocarbons in which F replaces hydrogen are characterized by a remarkable lack of toxicity This is due to the tight C—F bond. The plastic Teflon (called "Fluon" in England) becomes toxic only when heated above 300 C. It yields a toxic product called perfluoroisobutylene (18)

A group of fluorochloro compounds, better known as Freons (CCl_2F_2) which are used extensively as refrigerants and expellant gas are nontoxic. When heated, however Freon gives off such highly toxic products as hydrogen fluoride, hydrogen chloride and phosgene (19)

Certain polymers made from chlorotrifluoroethylene, mainly oils, greases and solids, such as Fluorolube and KelF are also inert.

Among other organic fluorides, the alkylfluorocarbons have received much attention because of their anesthetic properties. Some of them, especially tetrafluorobutane, trifluoropropane and trifluoroethane are very toxic. The unsaturated and asymmetrical fluorocarbons are the ones most likely to produce injurious effects.

Carbon tetrafluoride is remarkably free of toxicity in distinction to carbon tetrachloride, a treacherous poison. This difference between fluorides and chlorides is due to the fact that the C—F bond is much stronger than the C—Cl bond (20) If hydrogen in methane is replaced by chloride, the products become increasingly more toxic.

2. Inorganic Compounds

Toxicologically the inorganic compounds are of major concern as causes of acute and subacute intoxication.

For practical purposes, Roholm (8) distinguishes four groups

1 *Gaseous F compounds* such as the very toxic hydrogen fluoride (HF) and silicon tetrafluoride (SiF_4)
2. *Solutions* of hydrofluoric acid (HF) and hydrofluosilicic acid (H_2SiF_6) which are extremely toxic. This group includes acid solutions of fluorides and silicofluorides bifluorides in substance.
3 *Easily-soluble* fluorides and fluosilicates, which have a high degree of toxicity sodium fluoride (NaF) potassium fluoride (KF) ammonium fluoride (NH_4F) sodium fluosilicate (Na_2SiF) potassium fluosilicate (K_2SiF) and ammonium silicofluoride (NH_4F_2SiF)
4. *Almost insoluble* F compounds, whose toxicity is moderate or low Cryolite (Na_3AlF_6) calcium fluoride (CaF_2)

There are distinct differences in the action of different inorganic F compounds as well as in the tolerance of various animals to the drugs.

Comparative Toxicity of NaF in Different Animals Table II compiled from data presented by Roholm (8) and by Stokinger (21) presents the minimal lethal dose of NaF in various animals, by different modes of F intake.

Much of our present day concept of F toxicity is based on the observations with sodium F covered in table II

In dogs and rabbits, Roholm (8) the most reliable authority on the subject, recorded 23 to 45 mg of F/kg as the minimal lethal dose when given intravenously 23 to 90 mg orally This slight difference between the two suggested to him rapid

Table II Minimal lethal dose of NaF in experimental animals

Author	Year	Dose (mg/kg)	Animal	Mode of administration
Goldemberg (22)	1930	73	Rabbit	Oral
Wieland u. Kurtzahn (23)	1923	45	Rabbit	Oral
Schulz (24)	1937	200–400	Rabbit	Subcutaneous
Blairot (25)	1893	95	Rabbit	I. V
Leake (26)	1928	34	Rabbit	I. V
Stokinger (21)	1949	24–47	Rat	Oral
Goldemberg (22)	1930	13–16	Rat	Intraperitoneal
Leake (26)	1928	25–45	Dog	Oral
Schulz (24)	1937	300	Dog	Subcutaneous
deNito (27)	1928	14–25	Dog	Intramuscular
Perret (28)	1898	35	Dog	I. V
Magenta (29)	1928	25	Dog	I. V
Leone et al. (30)	1956	0.036–0.5	Dog	I. V
Wieland u. Kurtzahn (23)	1923	214	Frog	Parenteral
deNito (27)	1928	900–1,360	Frog	Parenteral
Leone et al. (30)	1956	46.0 ± 1.8	Mice	Oral
Leone et al. (30)	1956	23.0 ± 0.9	Mice	I. V

These data are very inconsistent and indeed confusing. It appears that frogs are most resistant among laboratory animals. Parenteral administration, of course, causes intoxication more readily than oral doses.

Table III Lethal dose of NaF in domestic animals

Author	Animal	Dose (mg F⁻/kg body weight)
Gadzhiev (32)(1955)	Calf	45.2
Habermann (33)(1945)	Sheep	86.6 and 129.8
Habermann (33)(1945)	Goat	93.5–199
Levenshtein and Dubrovin (34)(1947)	Horse	>45 g/kg

absorption of F⁻ through the gastrointestinal mucosa, a fact later confirmed by radioactive tracer studies (31).

In domestic animals the lethal doses of NaF are presented in Table III. There is no explanation why horses can tolerate so much more F⁻ than other species of animals. Additional data on the variation between species of animals in their resistance to F⁻ intake are presented in table IV.

Table IV Variation between species of animals in their resistance to F⁻ intake

	Minimal "tolerated"* dose (mg/kg)		Minimal toxic** dose (mg/kg)	
		as F		as F
Rabbits	147 NaF	54	200 NaF	73
Rats	94	34	125	46
Rabbits	100 Na_2SiF_6	61	125 Na_2SiF_6	46
Rats	50 Na_2SiF_6	31	70 Na_2SiF_6	42
Rats	150 $BaSiF_6$	61	175 $BaSiF_6$	71

* Killing less than 20% of the animals.
** Killing the majority of the animals.

Toxicity of Different Compounds. Muhlberger (35) compared the toxicity of NaF with that of Na_2SiF_6 and $BaSiF_6$ (barium silicofluoride) in rats and rabbits.

Table V Comparative toxicity for rats of six compounds of fluoride (according to Smith and Leverton (36))

Material	Days of survival	Average daily intake (mg/kg)
NaF	9	40
NH_4F	9	43
BaSiF	10	48
Na_2SiF	10	42
Na_3AlF	10	1,928
CaF	11	3,400

He found Na_2SiF_6 more toxic than NaF The difference in toxicity can partially be explained by the fact that NaF contains less F namely 45.25 per cent, Na_2SiF_6 60.54 per cent.

Relatively insoluble, BaSiF (barium silicofluoride) about equals NaF with respect to its toxicity when fed at the same order of magnitude.

Additional comparative data are available in subacute intoxication with various F compounds in Table V by Smith and Leverton (36)

Calcium fluoride and cryolite are much less toxic than the other compounds included in table V The effect of a daily intake of approximately 40 mg/kg of F from NaF is similar to that of 1,900 mg/kg cryolite and 3 400 CaF_2 (36) In these experiments F was added to food which renders it less toxic than when administered in water or as dry powder In chronic intoxication, under certain conditions, a reversal of the comparative toxicity between CaF and NaF seems to occur Fleming and Greenfield (37) found CaF_2 more toxic than NaF in fetuses of mice. Rabbits are fatally poisoned by cryolite administered orally in doses of 9—12 mg/kg, whereas rats can survive doses as large as 60 gm/kg (38)

Table VI Lethal doses in adult guinea-pigs (according to Simonin and Pierron)

Compounds	Oral (mg/kg)	Subcutaneous (mg/kg)
NaF	230	400
KF	250	350
NH_4F	150	250
LiF	200	2,000
MgF_2	1,000	3,000
CaF	> 3,000	> 5,000
SrF	> 5,000	> 5,000
BaF	350	350
AgF	300	800
SbF	100	200
MnF	700	700
Cr fluoride	150	120
CdF	150	200
PbF	4,000	> 5,000
ZnF	200	100
AlF	600	3 000
Ce fluoride	3,000	5 000
HF acid	80	100
Na bifluoride	200	250
K bifluoride	150	250
NH_4 bifluoride	150	200
H_2SiF	200	250
Na_2SiF	250	500
K_2SiF	250	300
$(NH_4)_2SiF$	150	200
MgSiF	200	400
CaSiF	230	450
ZnSiF	100	200
$Al_2(SiF)_3$	5,000	4 000
K-fluotitanate	200	450

Death in 48 hours.

A comprehensive listing of the toxic dose of various F compounds in guinea pigs is presented by Simonin and Pierron (39) in Table V

Guinea pigs can survive doses of CaF_2 as high as 5,000 mg/kg given either orally or subcutaneously Strontium, Cerium, Magnesium and Aluminum Compounds are less toxic than most other F salts. Simonin's data were based on some 2,500 tests.

Factors affecting F toxicity In long term studies, numerous factors affect F toxicity Some of these factors are likely to be significant in acute intoxication as well.

Gastric acidity renders CaF_2 more readily absorbable (40) and therefore more toxic.

Administration of *other elements* simultaneously with F has a distinct effect upon its toxicity For instance, addition of Al and Mg salts (41 42) Ca^{++} and P^{+} (43) interfere with F uptake and thus reduce F toxicity

When *associated with organic matter* F is less readily assimilated Food-borne CaF_2 less than water-borne (44) F in milk less than in water (45)

Young, growing animals retain more F in the skeleton than mature ones (46) Young dairy cattle develop fluorosis in pastures where mature stock is not affected (47) Older rats, however are more susceptible to acute intoxication than younger ones (48) Only half as much NaF is required to kill the average rat weighing 200 to 300 gm as a rat weighing 100 to 200 gm (21)

Synthetic cryolite is considered more toxic than natural cryolite (49)

Fat in the diet enhances retention and toxicity of F (50 51) Administration of thiouracil and thyroid gland has a similar effect (52)

During *pregnancy and lactation* there is greater retention of F (53) All these factors which are significant in chronic intoxication must be considered in acute intoxication as well.

3 Gaseous Compounds

The effect of gaseous F compounds on the animal organism is determined mainly by the length of exposure to the gas and by its concentration in the air i.e. the magnitude of F reaching the system through the air passages (21)

Hydrogen Fluoride (HF or H_2F_2) a colorless gas which condenses to a liquid at 19.5 C, is clinically the most important F containing air contaminant. Because of its large scale use in industry it is probably the greatest single air-contaminant hazard. It has a strong affinity to water and forms a mist with water vapor in the air Its toxic effect equals that of HCl and SO_2 (54)

When Machle et al (54) exposed rabbits and guinea pigs to HF at 1.0 to 1.5 mg/l of air they produced death in as short a time as 5 minutes. Concentrations of 0.79 mg/l for 5 minutes revealed marked pathology 23 hours to 55 days later mainly damage to respiratory tract although the animals did not die. Below 0.1 mg/l HF was tolerated for 5 hours without severe injury At 0.0245 mg/l animals exposed for 41 hours exhibited no outward evidence of illness.

Stokinger (21) in his classical inhalation experiments studied the effect of pure fluorine gas (F_2) of HF and NaF (as particulate) in rabbits, guinea pigs, rats and mice.

Elemental Fluorine Gas At a concentration of 200 ppm by volume after 3 hour exposures F_2 caused 100 %[1] fatality at 100 ppm the mortality was 54 % for rats, 96 % for mice.

The animals were exposed for 170 hours to much lower concentrations, namely 0.5 to 16 ppm (or 0.8 to 25 mg/m³) At the 16 ppm concentration F_2 caused a mortality of 75 % At the 0.5 ppm level the mortality was 4 % Dog rabbit and mouse were more susceptible than rat, guinea pig and hamster

[1] Except in guinea pigs for which the fatality rate was 90 %

F_2 is more toxic than HF. For HF the tolerated dose was 7 ppm, for F_2 only 1 ppm. This is the equivalent to 6 mg and 1.7 mg, respectively, of F ion per m³.

There are other toxicological differences in the two gases, F_2 and HF (21). Rats and mice are very susceptible to HF, dogs and rabbits less so. On the other hand, dogs and rabbits die from concentrations of F_2 which do not kill rats and mice.

According to Stokinger less F is retained by animals exposed to F_2 than to an equivalent concentration of HF. Both gases are highly toxic to the respiratory system.

Boron trifluorid (BF_3) is another very toxic F gas. If allowed to escape into the atmosphere, it unites rapidly with moisture to form a white fog, highly irritating to skin, eyes and particularly lungs. When rats, rabbits, mice, guinea pigs, dogs and cats were exposed 30 days for 6 hours daily (55, 56), all animals died at 100 ppm, only a few at 15 ppm. Pulmonary changes predisposing the animals to pneumonia and marked renal tubular degeneration were found in rats. There was a decrease in organic phosphorus prior to death.

Toxicity studies have recently been reported (57) on two extremely toxic gases — *selenium hexafluoride* (SeF_6) and *tellurium hexafluorid* (TeF_6). Their inhalation toxicity surpasses that of F_2, HF, SeH and TeH_2. TeF_6 is five times as poisonous as SeF_6 (54).

Among other F compounds sulfur hexafluoride (SF), an odorless gas, is physiologically inert. However, Virtue and Weaver (58) observed slightly narcotic symptoms in humans, pulmonary edema in rats and mice. The Threshold Limit Value according to Kimmerle is somewhat of the order of 0.025 ppm.

Table VII. Threshold limit values for F compounds for 1961

	PPM
Recommended values	
Gases and vapors	
Boron trifluoride	1
Chlorine trifluoride	0.1
Dichlorodifluoromethane	1,000
Dichloromonofluoromethane	1,000
Fluorine	0.1
Fluorotrichloromethane	1,000
Hydrogen fluoride	3
Sulfur hexafluoride	1,000
Sulfur pentafluoride	0.025
Trifluoromonobromomethane	1,000
Dusts, fumes and mists	
Fluoride	2.5
Sodium fluoroacetate (1,080)	0.1
Tentative values	
Teflon decomposition products (as F)	0.05
For comparison	
Hydrogen chloride	5
Sulfur dioxide	5
Chlorine	1

Other Threshold Limit Values for F Compounds are presented in Table VII.

B. Acute Intoxication in Animals

In order to evaluate the clinical picture of acute F intoxication in humans, a review of intoxication in animals is in order.

1. In Experimental Animals

The German pharmacologist, Tappeiner (60), presents one of the most reliable descriptions. He carried out the first systemic investigation on the subject in warm-blooded animals (rabbits, guinea pigs, mice, cats and dogs).

With 0.5 gm NaF per kg orally and 0.15 mg/kg subcutaneously he observed in two dogs sopor profound general weakness, convulsions involving parts of the body or the whole system. There was increased frequency and depth of respiratory movements vomiting, salivation and lacrimation which failed to respond to atropine therapy This was followed by paralysis of the respiratory and vasomotor center He observed early appearance of rigor mortis.

In intoxication in rabbits with a 2 % aqueous solution of NaF given intravenously Blaizot (25) related the following symptoms

After *50 mg*/kg No symptoms except for increase in appetite.

After *80 mg*/kg (36 mg F) Dyspnea, moderate salivation, slight fever 2 to 3 hours later the animals fully recuperated.

After 100 mg/kg (45 mg F) Intensive dyspnea, extreme salivation, polyuria, fever and polydipsia. 10 to 15 minutes later the hind portion of the animal became paralyzed extensive fibrillating, muscular contractions and trembling was noted. Gradually the animal died in coma.

In Muehlberger s (35) experiments on rabbits and rats a single toxic dose (see Table II) caused salivation, diarrhea, trembling with terminal clonic and tonic convulsions. Some animals survived 5 to 7 days they died of cachexia.[1]

Roholm (8) demonstrated the effect of nonlethal doses of NaF on muscles of frogs. He observed persistent fibrillation of all striped muscles lasting for 24 hours. When he increased the doses, the muscles became rigid.

In inhalation e… ments (21) with pure F gas, HF and NaF in rats and guinea pigs, acute respiratory symptoms dominated the clinical picture. After F_2 exposure the fur became coarse and stiff the eyes, nasal and buccal mucosa irritated, especially in the rat. "Irrational seizures" occurred in the dog, followed by death. Outward signs of toxicity are often absent. In subacute exposures to HF extending over 166 hours at the 25 mg per m^3 level, Stockinger noted in some animals a persistent weight gain which is followed by loss of weight prior to death. In addition to pulmonary irritation, the rat and dog may show testicular degeneration.

2. Domestic Animals

Most data on domestic animals are confined to chronic intoxication (61) Here, the toxic dose in chronic poisoning varies with the state of growth, development and reproductive processes (62) This undoubtedly holds true in acute intoxication as well. Among domestic animals cattle and sheep are most susceptible to fluorosis pigs and horses more resistant fowl appears to be most resistant.

NaF administered orally to farm animals experimentally produced the following symptoms Loss of appetite and weight, diarrhea, general malaise, evidence of abdominal distress, salivation (8, 33 63 67)

Animals which swallowed accidentally large quantities of Na_2SiF_4 in their diet (64 65) exhibited salivary frothing, chewing motions, dilated pupils, unsteady locomotion, muscular weakness, loss of balance, shallow respiration oliguria and convulsions.

On farms F containing superphosphate fertilizer taken accidentally by animals … duced (66) polydipsia salivation lack

[1] At …utopsy these an… …ed changes in …ey and liver altho… …ally there were …ptoms cl… damage to any …ular organ.

of appetite, evidence of pain in abdomen, injection of conjunctivae, hyperemia in the mouth, a tendency to diarrhea with blood and mucus. The animals lay with extremities extended and spread widely tonic convulsions developed later

From F containing phosphate used as supplementary feed in animals acute intoxication has been reported, manifested by diarrhea, loss of body weight, depression and general weakness (35 63) Vomiting rarely occurs. Abdominal distress, salivation, decreased milk production in goats (68) glycosuria in sheep (69) tremor and vomiting in swine (33) occur

A horse which had ingested daily for three days 3 4 to 3.8 gm of F present as a contaminant of calcium phosphate (70) developed a weakened pulse and an hour long episode of colic, diarrhea, hemolysis. Blood coagulation time was prolonged.

Pigs which consumed daily 304 to 380 mg (10) of F in calcium phosphate — containing 8 4 % of NaF as contaminant, — showed muscular weakness and subsequent paralysis. Inflammation of the visible mucous membranes and evidence of spastic constipation was noted.

The principal features of acute intoxication in domestic animals, therefore, are gastroenteritis, convulsions, muscular and respiratory paralysis

C. Acute Intoxication in Humans

There are three modes of acute F intoxication in humans

1 Through oral administration.
2 Through inhalation.
3 Through local contact.

The portal of entry of the poison determines largely the symptomatology There is primarily involvement of the gastrointestinal tract in oral intoxication of the respiratory tract in inhalation of F of the skin after local contact.

1 Ingested Fluorides

The first fatality from F was reported in 1873 by King (71) A 35-year-old male died within 35 minutes after he had taken about 15 cc of hydrofluoric acid. Up to 1935 Roholm (8) recorded 112 cases among which 60 (53 6 %) terminated fatally The ages ranged from 2.5 to 76 years males and females were equally represented. The bulk of the cases were accidents due to mistaking F for another substance because of its close resemblance to salt, flour and sugar in households. F happened to be in the house because it had been employed as insect powder rodent killer disinfectant and preservative. Specifically it was mistaken for sodium bicarbonate (72) (73) powdered sugar (74) cornstarch (75) baking powder (76) (77) Epsom salts (78) Rochelle salt (76) (77) "laxative salt (75) and powdered milk (79)

The 112 cases included several suicides and homicides. In 52 the F compounds were known, namely NaF in 27 Na_2SiF_6 in 8 hydrofluoric or hydrofluosilic acid in 15

a. *Manifestations*

Acut F intoxication is probably much more prevalent today than would appear from the literature. As recent epidemic revealed (80) medical authorities are inadequately informed on this subject. The disease can be mistaken for botulism, ptomaine poisoning" intestinal flu etc. It thus escapes attention of the profession.

Epidemics: During the past 30 years, several mass poisonings have been reported. The worst one on record (79) occurred in 1943 at the Oregon State Hospital at Salem, fol-

lowing a meal of scrambled eggs. 163 inmates took ill, 47 of them fatally. The identity of the poison, a roach powder containing 90 % NaF was not established until approximately 22 hours had elapsed. A helper in the kitchen had mistaken roach powder for powdered milk. He had added approximately 17 pounds of the compound to a 10-gallon mixture of scrambled eggs to be served at an evening meal. Many of the victims rejected the food because of its salty soapy taste. Soon complained of numbness of the mouth as soon as they ingested it.

In 1935 14 cases occurred in Kiel, Germany 2 of them fatal. They had ingested a cake purchased in a bakery where Na_2SiF_6, mistaken for powdered sugar was added to the cake (81). The disease was at first diagnosed as botulism. The symptoms severe vomiting, spastic abdominal pains, tonic and clonic convulsions, spasm of pharynx and esophagus — particularly when attempts were made to lavage the stomach — ptosis, strabismus, sluggishly or non-reacting pupils, diplopia, extreme thirst and headaches. There was erythema and edema of eyes and face, presumably due to the local caustic reaction from the vomitus.

In 1936, 21 persons were stricken, 3 of them fatally with intoxication by a mixture of sodium bicarbonate with 4 % NaF and 44 ppm arsenic sold as baking soda (72). In the fatal cases 0.5 to 1 teaspoon was taken in water. In eight nonfatal cases it was used in pancakes.

A milder outbreak of mass F poisoning occurred in December 1939 in a New York State reform school (82) in boys aged 12 to 22. After eating chocolate pudding, 69 out of 97 students developed gastroenteritis, nausea, vomiting, abdominal cramps and later 1 to 2 loose bowel movements, weakness, sweating, headaches, salivation and lacrimation. There was no fever no blood in vomitus or feces. Most boys were seized within 10 minutes of finishing their dessert, in one case the time interval was greater than 1/2 hour in another 2 1/2 hours. All recovered promptly within a few hours except for residual weakness, slight anorexia, vague epigastric uneasiness and soreness which lasted for several days. Someone in the kitchen had deliberately added roach powder to the pudding. Approximately from 0.2 to 0.6 gm of NaF far less than that considered the fatal dose (5—15 gm) was ingested per person. 27 inmates who consumed the pudding had no symptoms whatsoever. Some of them were believed to have taken less than 0.2 gm NaF. Large amounts of milk used in preparing the pudding may have acted as a partial antidote and thus averted more serious consequences.

More severe was an outbreak in a Pittsburgh, Pennsylvania, Salvation Army Center in 1940. NaF mistaken for flour had been added to pancakes. It resulted in intoxication of 40 persons and 12 deaths (83).

In 1938, 280 workers (80) in a German factory developed within half an hour after a meal burning pain in the epigastric area, diarrhea, fever and severe vomiting. The epidemic was of minor severity none of the workers was disabled from work. A baking powder added to pears consisted of 61 % NaF 39 % sodium bicarbonate. In addition, it contained traces of iron, calcium and sulfate. Each portion per person was estimated to contain 0.7 gm of NaF. It was believed that during the preparation of the meal some NaF was turned into CaF_2 thus considerably diminishing the toxicity.

Subsequently two persons took experimentally 2 gm of the powder (1.2 gm NaF) by mouth. It had an unpleasant bitter taste. In 10 to 15 minutes they reacted with salivation, burning sensation in the stomach and esophagus, slight cramp-like pains and marked diaphoresis. After 20 minutes, one person experienced nausea and vomiting; the other vomited severely and continued to be ill for approximately 3 days with vertigo, loss of appetite, burning in the stomach.

In April 1946 (84) acute poisoning occurred in 34 persons, 19 male, 15 female in a north German city. All experienced nausea, abdominal pain, vomiting a few hours after eating bread which was contaminated with F containing flour. Some had epileptiform convulsions, one severe coma. All recovered with no sequelae following immediate gastric lavage and intravenous administration of sodium luminal. The amount of F in the flour was not determined.

Between 1947 and 1949 the coroner's office in Cincinnati (85) autopsied 6 cases, 4 of which were suicides, 2 accidental. In one family the husband expired, four other mem-

bers recovered. The flour used in preparation of the last meal contained 4.56 mg F per 100 gm of flour

According to Bredemann (L.) in 1953 a number of children took ill after eating hard candy suckers obtained t county fair Analysis showed that some contained 410 ppm of N F another sample 23,800 ppm.

In 1937 of 99 persons who ate pricot preserves in a Berlin restaurant (86) 59 developed gastroenteritis, 53 recovered the next day 6 after 2 days. In lunchroom 42 of 56 persons who had eaten a similarly prepared pudding took ill. Analysis of two samples revealed 180—400 ppm of F in the apricot preserves, 177 ppm F in the pudding It was estimated that a person could have ingested 66 mg of F from this pudding.

In December 1961 (87) at a Rotary dinner in St. Johns, Michigan, some 60 persons took ill, none seriously S bsequently it was learned that the cook had used by mistake NaF instead of sodium bicarbonate for some cakes. When sh became ill she ttempted to relieve her upset stomach by taking a teaspoon of what she presumed was sodium bicarbonate. This dose was fatal to her after 8 hours (see Case 3)

The occurrence of these epedemics, summarized in Table VIII, demonstrates that this type of poisoning represents significant health problem. The question arises as t how many more such epidemics occur without being brought t the attention of the medical profession either because they remain un diagnosed or because physicians fail to report such incidents under the impact of litigation and of political considerations.

Individual Case Reports A few pertinent cases illustrate certain features of the disease. They also demonstrate the diffi culties encountered in securing proper laboratory and clinical data because of the rapid course of the disease and lack of prompt recognition.

Two Phases of the Disease I Maletz case (74) there were tw distinct phases of the disease, a feature observed by Lidbeck (79) as well. A 41-year-old had partaken of a batch of hard sauce which consisted essentially of butter and sugar contaminated with roach powder Within less than an hour he was overcome by weakness, nausea and vomiting, excess perspiration and shock. Gastric lavage and administration of 60 cc of magnesium sulphat and saline purgative induced considerable improvement. Nausea and omiting subsided.

Several hours later a second stage developed with generalized muscular cramps, partic ularly of the face. At first this improved. Six hours after onset, while ttempting to talk, he suddenly experienced shortness f breath, dyspnea, and expired.

R covery after a Massive Dose Persistent and severe omiting which liminated large portion of the chemical was probably responsible for recovery of a 16-year-old pregnant schoolgirl (88) She had taken for suicidal purposes one-third to one-half of a drinking glass of roach powder in water estimated to epresent 50—80 gm of NaF an unusually large dose. One hour later she exhibited severe abdominal cramps and vomiting, involuntary watery diarrhea and a temperature of 99.6. There was evidence of a burn at sites of contact with vomitus on face, neck and chest ("vomitus burn) profuse salivation, injection of the pharynx, shallow and slow respiratory movements, loud coarse tracheal rhonchi, tender abdomen, grossly audible peristaltic rushes at 1 to 15 minute intervals. The respiratory rat slowed down to 12 per minut the blood pressure normal t first, f ll gradually

Four and one half hours later she developed Chvostek and Trousseau signs, paresthesias in extremities. The vomiting continued. Pieces of tissue 1 × 1.5 cm proved to be plates of mucosal epithelium, presumably gastric. A specimen of the vomitus sealed and stored in the refrigerator was subsequently found t have etched th glass ial in which it had been placed. Following large doses of calcium, she gradually improved and recovered completely During her pregnancy she showed only slight anemia. In the child born at term no abnormalities were evident.

Allergic Reaction A nurse who had taken 11 gm of sodium fluorosilicat (89) had what appeared to be an allergic type of reaction to the drug Vomiting occurred only once. 3 hours later universal urticaria appeared which was now accompanied by continuous vomiting

Table VIII Epidemics of acute intoxication

Authors	Place	Morbidity	Fatalities	Compound	Approximate dose	Sources	Symptoms
Heydrich (81) 1935	Kiel, Germany	14	2	Na_2SiF	?	Added to cake instead of sugar	Convulsions; Headache; Polydipsia; Eye muscle palsy; Delayed blood coagulation
Geiger (12) 1936	San Francisco	21	3	NaF (74 %) + Na_2CO + As (44 ppm)	0.5–1	Added to pancakes	Diarrhea
Griebel et al. (86) 1937	Berlin	59 out of 9[illegible] 49 out of 56	0	HF	60 mg F	Preservativ in apricot pudding	Diarrhea
Ingraham et al. (82) 1939	New York City	69 out of 96	0	NaF	0.2–0.6 g	Roach powder in chocolate pudding	Diarrhea Salivation Cephalgia
Anonymous (83) 1940	Pittsburgh, Pa.	40	21	NaF	–	NaF mistaken for pancake flour	Chill; Vertigo; Muscular fibrillation Convulsions
Lidbeck et al. (19) 1943	Salem, Oregon	263	47	NaF	–	Roach powder in scrambled eggs-17 lb. in 10 gal. of eggs	Carpopedal spasm; Urticaria Dyspnea
Guttreit (84) 1946	Germany	34	0	NaF	–	Bread	Convulsions
Cleveland (85) 1947–49	Cincinnati, O.	5	1	?	–	Flour	–
Dungahn (80) 1958	Germany	280	0	NaF	0.7 g	"Baking powder" added to pears	Diarrhea Fever
Bredemann (12) 1953	Germany	–	0	NaF	41 mg % 2,560 mg %	Contaminated hard candy suckers	–
Black (81) 1961	St. Johns, Mich.	About 40–50	1	NaF	–	"Baking powder" in banana cakes	–

Other than nausea, vomiting, abdominal cramps.

of hemorrhagic material, abdominal pains and diarrhea, a sensation of suffocation.

Six hours later vomiting ceased. Tetaniform convulsions in both hands, especially the ulnar portions, were accompanied by spastic contractions of eye muscles and paresis of extensor muscles of the hands. She expired 10 hours later during convulsions.

Dominant Neurological Features. A 49-year old woman (90) had taken about a dessert spoonful (5.5 gm) of NaF used as dusting powder for poultry in the belief that the tin contained sulfur only. Besides vomiting and diarrhea, there was ptosis of the right eyelid and right external strabismus. Complete recovery took place within 18 days.

Intoxication in Child. A 3 1/2-year-old girl swallowed a pellet of rat poison believing it to be candy (91). The amount of Na_2SiF_6 ingested was about 0.52 to 0.7 gm. Death occurred after 3 hours inspite of the fact that considerable dilution of the poison was induced through gastric lavage. Since the child had taken water shortly after the poison, it is likely that much of it reached the intestinal tract before lavage could be instituted.

Absence of Gastric Symptoms. In the suicide of a 59-year-old man reported by Rabinowitch (92) from an unknown dose of NaF there was no vomiting. Though in shock, he remained fully conscious. At first, carpopedal spasms in feet and hands, later tetaniform convulsions developed. Death occurred 3 hours after ingestion of the poison. At autopsy only a single "small lesion" of the gastric mucosa was found near the duodenum. This limited involvement may have accounted for the absence of vomiting.

Protracted Fatal Case. A protracted fatal case due to 17.5% solution of HF is reported by Fanke (93). The 53-year-old male survived for 12 days after he had taken about a teaspoonful of the solution. Within a few minutes he vomited severely and became unconscious. After gastric lavage and cardiac stimulants, he improved. Five days later he became lethargic and developed spastic pains in the hypogastrium, hoarseness, tachycardia, irregular pupils, anuria. The *EKG* was normal. Blood pressure 160/110. He expired after 12 days with what appeared to be a cardiac death. At autopsy there was evidence of myocardial damage.

Intoxication with Fluoroacetate. Harrison (94) reports a fatality from fluoroacetate (a delayed convulsant) in a 40-year-old male. The dose was unknown. The symptoms were unconsciousness, nystagmus of both eyes and muscular spasms. This was followed by an epileptiform convulsion, in spite of gastric lavage and instillation of magnesium sulphate. Death occurred 17 hours later with muscular spasms, profuse perspiration and flushed face. The blood pressure had risen slightly prior to death.

Another instance (95) a 2-year-old negro boy licked a bottle containing 1080. Persistent vomiting was followed after 6 hours by tetanic convulsions, irregular breathing, coma. Calcium gluconate controlled the tetanic convulsions, but tonic convulsions occurred for 2 days and respiratory paralysis necessitated artificial respiration on the 3rd day. The patient gradually recovered on the 6th day.

The striking feature in all these cases is the appearance of gastroenteritis at first, of certain neurological manifestations later. Only in the Rabinowitch (92) case were the initial gastric symptoms absent. Death seems to be precipitated either by shock in the initial stage or by the neurological and cardiovascular manifestations, during the second stage of the disease.

Unreported Cases. The two following cases have come to my attention through the courtesy of Dr. A. V. Arms, Kansas City St. Lukes Hospital.

Case 1 (W.F.R.) A 43-year-old white male on 10—14—59 exhibited severe vomiting, nausea, cramp-like pain in the abdomen, shock and syncope. Three hours previously he had taken a grape drink which contained NaF. He died within 10 minutes after admission.

The lungs showed congestion in the dependent portions; the bronchi contained mucoid material, there were petechial hemorrhages in the visceral pleura. The stomach exhibited patches of severe hyperemia and hemorrhagic areas at the summit of the rugal folds (Fig. 1). The small bowel and colon showed marked distention, the liver severe vascular congestion, the kidneys distention of tubules and flattening of the cells lining the tubules (Fig. 2). No other pathology was found.



Fig 1 Gastric mucosa in acute F poisoning; Case 1 — 42-year-old female, homicide. Note Cellular infiltration, loss of surface epithelium; obliteration of normal glands. (Courtesy of Dr. A. V Arma, St. Luke' Hospital, Kansas City Mo.)

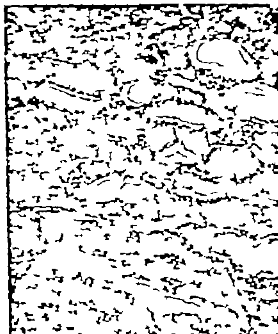


Fig 2. Kidney section, Case 1 Note Dilatation of tubules; flattening of epithelial cells. (Courtesy Dr A. V Arma.)

Case 2 (E.R.R.) A 4 -year-old white female dmitted simultaneously under the same ircumstances, was moderately coherent and answered questions for about 15 to 20 minutes. She omited bloody mucus. Gastric lavage showed no return through the tube. The blood pressure rose t 182 following intravenous fluids and solucortef, but fell shortly thereafter t shock levels. Administration of calcium and levoled failed to maintain blood pressure. The patient died in deep cyanosis, dyspnea, cardiac arrhythmia and deviation of the ey muscles

At autopsy there as cyanosis of the skin, patches of intense hyperemia in the gastric mucosa near the fundus and a shallow hemorrhagic ulcer near the cardia, 1 cm in diameter. There was moderate congestion in the lungs and liver

These two cases, husband and wife, represented homicide by their daughter who had dded an unknown amount of NaF to the grape drink.

Through the courtesy of Dr C. E. Black of Lansing, Michigan, I obtained the following case report



Fig. 3. Gastric mucosa in Case 3, 69-year-old female accidental F poisoning. Note: Extensive necrosis of the gastric mucosa, destruction of gastric lining, destruction of gastric glands. (Courtesy Dr C. E. Black, Lansing, Mich.)

Case 3 Mrs. L. G 69-year-old white female cook, became acutely ill t 1:30 P.M. December 19 1961 with abdominal cramps, nausea, vomiting and severe diarrhea. At 5 00 P.M. she was hospitalized in shock and was placed in oxygen. Unable to speak, she could hear and answer questions by nodding and shaking her head. The pulse rat varied from 50—160 per minute. Respirations were qually as erratic, especially when oxygen was withdrawn for a minut or two. The pupils wer dilated, reacted t light and ccommodation. There was no excoriation on lips, tongue and buccal mucous membranes. Blood pressure was 130/98, the pulse irregular in force and rhythm. The right elbow reflex was present. The abdomen was not distended t this time; some tenderness was elicited without clear-cut tendency of localization. There was severe dyspnea and tremor She expired with increasing dyspnea at 9 00 clock.

The initial tentative diagnosis had been Cerebral Vascular Accident.

At autopsy the only unusual feature was th presence of 400 cc f hemorrhagic material and partially digested food in the stomach. The stomach content etched glass. There were widespread hemorrhages throughout the mucosa, submucosa and the wall of the stomach (Fig. 3) Small and large intestines appeared normal. No hemorrhages were present elsewhere. N characteristic odors were detectible.

The diagnosis was acut hemorrhagic gastritis, accidental sodium fluoride poisoning

The following two cases represent acute intoxication precipitated by sudden intake of excessiv amounts of F

Case 4 (Mr R.O.) a 32 year-old farmer was hospitalized on 3/10/57 because f acute episodes of bdominal pain radiating int both flanks, persistent omiting, nausea severe headache, pain in the lower back, dysuria, blurred vision, nd severe exhaustion. These seizures were always associated with and followed by extreme muscular weakness and lethargy

H had always been in good health until he moved int farmhouse where the water contained 0.9 ppm (mg/liter) of F Between March 1954 and March 1957 he experienced 10 similar episodes. On several occasions they were accompanied by urticaria and followed by stomatitis. Diagnostic studies on previous hospital admissions had been to no vail.

The present episode originated within half an hour after spreading superphosphate fertilizer on the field. Besides phosphorus, it contained F A strong wind had blown much of the chemical int his face when loading it into the spreader

Toxicological studies by Dr E. W Kivela of the Michigan State Health Department onfirmed the diagnosis

Cases 4 and 5 have been recorded elsewhere (96)

A sample of the same brand of fertilizer contained 6 773 5 ppm or 0.68 per cent of F

On examination the blood pressure was 170/100 (later 128/75) pulse 86. There was right sided facial edema, marked increase in tendon reflexes, loss of abdominal reflexes, distention and tenderness of the abdomen, pain on palpation of the costochondral area, hypogastrium, and of the lumbar spine. Trousseau and Chvostek signs were negative, blood Ca 11.2 mg % albumin 4.8 globulin 2.4 alkaline phosphatase 6.2 (K.A.) units; acid phosphatase 3.2 NPN 37.5 BUN 12.6; PBI 3.2 E.S.R. below 3 mm/hr cephalin flocculation 4+ The 24-hour urinary specimen contained 350 mg phosphorus; 309.9 mg Ca^{++} 3.4 mg F

This condition subsided within 2 days without therapy he returned to work on the 3rd day following this episode.

Considering the fact that the previous seizures occurred in mid-summer only when he drank large amounts of water he was advised to obtain distilled water for cooking and drinking and t avoid high fluoride food such as fish, tea and gelatin. Subsequently he moved t another farm where the water supply contained only 0.2 ppm of F He has not had any recurrence of the disease.

After the patient had been completely well he was given on February 6th, 1960 double blind test with 15 mg NaF (6.8 mg F) using the same dose of NaI and NaBr as controls. Each salt was given dissolved in 300 cc of w ter w th 3-day interval between trials. Th solution f N F precipitated vomiting and spastic pains in stomach and upper intestinal tract, severe headache, lethargy paresthesias muscular fibrillation and urticaria. NaBr and N I were tolerated w thout ill effect

Case 5[1] Mrs W E.A., 62 developed abdominal cramps and distention, urticaria, allergic rhinitis and sinus disease, conjunctivitis dysuria and polyuria on five occasions within hours after arriving for visits in Washington, D C and Richmond, Va Each time it took up to 3 weeks for the condition t subside. E entually sh becam war that these cities has added F to their water supplies, hereas the water the drank at home was practically F free. On subsequent visits, by avoiding city water for drinking and cooking, recurrence of such episodes was eliminated.

7/14/56 an intradermal injection with 0.1% of an aqueous solution of NaF (0.1 mg) precipitated within 10 minutes the above symptoms which lasted about 24 hours. Control injections with horse serum, saline solution and with weaker aqueous dilutions of NaF had no adverse effect.

The patient remained well until gastric cramps, diarrhea, ulceration in th mouth and nasal symptoms recurred. She had been using a new toothpaste but was not aware that it contained F (approximately 1 mg of stannous fluoride with each brushing) Since the symptoms were identical with those which sh had suffered previously from drinking water in Washington, D.C. and Richmond and from the test dose, her ttention was drawn to F in toothpaste.

She again remained well until May 1960, when she w s given by her physician trifluoperazine (Stelazine) 1.0 mg 3 times a day On the second day she noted griping pain in the abdomen, flatulence, persistent nasal congestion, burning and pain on urination, backache, cephalalgia, marked lethargy and muscular weakness Her physician had not anticipated ill ffect from F contained in the drug (0.12 mg F per 1 mg tablet) The symptoms cleared within a week after discontinuing the drug

On 7/24/60 th disease was reproduced by double blind test instituted by D C D M. Cornstarch and Librium each taken for 1 eek, served as controls. At the height of the disease, blood Ca was 8.3 mg % phosphorus 5.6 mg % the 24-hour urinary F level 0.45 mg

Another double blindfold test was conducted subsequently This time her physician insisted on her taking the drug for 1 week following onset of symptoms. This resulted in severe disability from which it took 2 months to recover The major featur was intestinal pain, diarrhea, X-rays taken at this time showed spastic bowels with beginning diverticulosis, a condition which had not been present on previous examinations.

Th physician considered this a case of llergy t F In few of such symptoms as rticaria and typical allergic nasal disease.

[1] Cases 4 and 5 ha been recorded elsewhere (96)

b. *Diagnosis*

Symptomatology. From these reports it is evident that the manifestations and severity of acute F intoxication are determined mainly by the kind of F compound ingested, its dose, by the composition of the gastric content and the kind of food ingested simultaneously with the poison and, of course, by the antidote given. The extent and promptness of vomiting, i.e. ejection of the poison, is significant prognostically. There appears to be a tendency to allergic reactions in susceptible persons, a fact brought out previously in connection with chronic intoxication (9).

In the above cases three groups of symptoms prevailed: gastrointestinal, neurological and cardiorespiratory.

Gastrointestinal. Nausea, vomiting, burning, cramp-like abdominal pains, and diarrhea dominate the picture and are most consistent. Vomiting occurs abruptly, at times simultaneously with diarrhea. The vomitus and the stool show blood; excessive salivation and polydipsia prevail. Only rarely (92, 93) are gastrointestinal symptoms absent.

Neuromuscular. Usually after a short interval, tonic and clonic convulsions occur. They may be confined to arms and legs or affect the whole system. They are accompanied by muscular pain, fibrillation of muscles and occasionally by carpopedal spasm. There may be pareses of the affected muscle groups, especially eyes, face, hands and lower extremities, dysphagia and uncoordinated eye movements. As a rule the patients do not lose consciousness; there is no spontaneous defecation and urination as seen in epileptic seizures.

Cardio-respiratory. Cyanosis, tachycardia, hypotension, shallow irregular respiration, faint cardiac sounds, pallor, diaphoresis and thick mucoid discharge from the mouth and nose suggest cardiac and respiratory involvement. In a few cases (93) cardiac failure and pulmonary edema is reported. Extreme muscular weakness is a characteristic feature of the disease.

Less consistent are urticaria (72) (79) (89), fibrillary contractions of skeletal muscles (89) (97) (99) and subcutaneous hemorrhages. Severe headaches were reported in intoxication with NaF (100) and Na_2SiF_6 (82). During the course of the disease, fever may occur (101). Bell (90) observed right sided ptosis, external strabismus, diplopia. Heydrich (81) esophageal spasm.

Roholm summarized the symptoms in 34 cases of acute F intoxication (table IX).

Table IX. Roholm's classification of symptoms in 34 cases of acute fatal fluorine poisoning

	Cases
Vomiting	31
Pains in abdomen	17
Diarrhoea	13
Convulsions, spasms	11
General weakness, muscular weakness, collapse	8
Dyspnoea	7
Pains and paraesthesiae in extremities	6
Paresis, paralysis	5
Difficulties with speech, inarticulation	5
Thirst	5
Perspiration	5
Weak pulse	5
Change in facial color	5
Nausea	4
Unconsciousness	4
Salivation	3
Impaired swallowing	3
Motor restlessness	2
High temperature	2
Dizziness, headache, hiccough, urticaria, cold shivers, choking sensation, pupil contraction, uncoordinated eye movements, pains in sacral region, low temperature	1

Table X. Reactions in 5 out of 123 allergic patients given test dose of 15 mg NaF (6.8 mg F)

Patient	Sex	Age (years)	Date	Onset after	Duration	Symptoms
1 M. J	♀	57	8/16/56	min.	12 hours	Paresthesias Nausea; Vomiting Migraine Scotomata Lethargy
2 F L. P	♂	61	8/31/56	2.5 hours	10 days	Cephalalgia; Vertigo Tinnitus; Lethargy; Epigastric pain; Stomatitis; Blepharitis
3 H. S	♀	33	9/ 7/57	30 min.	48 hours	Facial edema; Cephalalgia; Vomiting Epigastric pain Spastic colitis
4 P O	♀	40	2/11/58	2 3 hours	7 days	Urticaria Pruritus; Arthralgia in spine and extremities; Cystitis; Paresthesias; Hyperreflexia
5. D S	♀	61	4/17/59	20 min.	10 hours	Nausea, Abdominal cramps; Cephalalgia Paresthesias Muscular fibrillation Stomatitis

Given 10 mg NaF (4.6 mg F)

Roholm, a biologist, although evidently not familiar with medical terminology describes vividly in this table the distribution of symptoms. Vomiting, abdominal pains and diarrhea constituted the principal feature of the disease neurological symptoms occurred in a smaller percentage of cases features of shock were not always conspicuous.

In susceptible, especially allergic, individuals extremely low doses may induce acutely toxic manifestations

A selected group of allergic patients suspected of F intolerance were given 15 mg of a freshly prepared solution of NaF (6.8 mg F) in 300 cc of distilled water 93 out of 123 had no ill effect whatsoever 25 experienced slight nausea 5 had symptoms noted in Table X.

In considering the extremely small dose of F employed in these studies, the wide variation in susceptibility from individual to individual is evident.

Onset of symptoms In most reported epidemics symptoms started within 30 minutes after ingestion of the poison. Lidbeck et al (79) furnish information on the onset of symptoms in relation to the amount of F detected at autopsy One patient who died 15 minutes after ingestion of the contaminated eggs, showed 17.2 gm of NaF in the stomach. The patient from whose stomach only 180 mgm. was recovered, survived for 18 hours (Table XI)

Differential diagnosis Gastric symptoms occur in acute intoxication from many corrosive poisons. The subsequent neurological features, especially the tetaniform convulsions without loss of consciousness, specifically point to intoxication with a calciprive chemical, particularly to oxa

late and citrate intoxication (8) In distinction from oxalate intoxication there is no acute kidney colic in acute F intoxication nor does the urine show the characteristic calcium oxalate crystals. Because of fever other kinds of acute gastroenteritis must be ruled out such as dysentery paratyphoid infection, botulism, etc. In these diseases, fever is a cardinal feature and occurs immediately at the onset. In F intoxication fever is less pronounced It does not occur at the beginning stage of the disease. That the disease can be confused with cerebral vascular accident is shown in Case 3

Some (102) have expressed reservations in accepting reports based on the clinical aspect of the disease. They point to the sparsity or absence of laboratory data and toxicological studies, such as F determinations in urine, tissue and blood which they consider requisites for establishing the diagnosis. Undoubtedly the most striking evidence of poisoning is the fact that the poison consumed is identified as F All other data, desirable as they are, are of secondary significance.

Laboratory findings Laboratory findings in acute fluorosis are sparse and somewhat confused. In a given emergency immediate therapeutic measures must necessarily take precedence over laboratory studies, which thus are liable to be neglected.

A crude but highly specific test is the etching of glass by the stomach content of the vomitus.

Most significant are Serum Ca++ levels. Rabinowitch (92) noted hypocalcemia as low as 2.6 % in serum taken a few minutes before the patient died This is the lowest value ever found in a human being In Maletz case (4) blood Ca++ after death was 5.8 mg % Peters (88) observed 24 hours after the intake of NaF a blood Ca++ level of 9 16 mg % and

Table XI Onset of symptoms in relation to F- recovered in stomach

	NaF found in stomach (g)
Within 15 minutes	17.2
Within 60 minutes	3.7
Within 240 minutes	1.06
Within 18 hours	0.18

1 liver and kidneys.

simultaneously a low blood phosphorus level of 1 7 mg %. Subsequently the blood Ca++ rose to 11.6 [1]

Leone et al (29) observed a slight drop in serum calcium in 9 out of 11 dogs given acutely lethal doses of NaF These determinations were made immediately before death.

In own studies 103 determinations of blood calcium and blood phosphorus were made on 81 patients before and after oral administration of NaF 15 mg (6.8 mg F) In a few cases there was a distinct response to the test dose (Fig 4) However in general, blood Ca and P levels 1 1/4 hours after the test dose were quite erratic. In 26 instances both values increased simultaneously in 9 they decreased. There was no correlation of Ca and P levels with the presence of symptoms nor with whether the patients were on a low or high Ca diet. The wide variations in F uptake and storage between individuals undoubtedly account for the inconsistent results.

In 4 persons who had taken 0.2 gm of NaF with barium carbonate, Davydov (103) observed leukopenia with relative and absolute neutropenia and relative lymphocytosis. Peters (88) however recorded a leukocytosis of 14,000 several

[1] Intravenous calcium gluconate may have contributed to this value

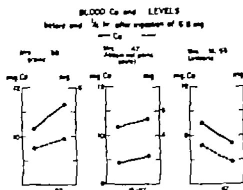


Fig 4. Blood Ca and P levels before and after ingestion of 6.8 mg F-, in three selected cases.

hours after intake of the poison. In Ma letz case the white blood count prior to death was 20,500 with 91 % polys, 65 % lymphocytes, 2 % monocytes and 0.3 % basophiles.

Fanke (93) related a sodium chloride level in blood serum of 673.9 mg %. His blood sugar values ranged between 90 and 333 mg % 5 days after the poisoning incident. It is not stated whether glucose had been given to his patient. Experi mentally in dogs (29) and in rabbits (104) hyperglycemia but no glycosuria has been observed.

According to Roholm (105) there is a tendency to reduction of hemoglobin and red blood cells. Lethal doses have been observed to be associated with retardation in blood coagulation (81)

One of my patients, Mrs F C K., aged 45 responded 10 minutes after an intra venous injection of 4 4 mg of NaF (2 mg of F) with a moderate toxic reaction, namely paresthesias in both arms, vertigo, dryness in the nose auditory disturbances. There was a decrease in the serum amylase (from 284 mg % to 264 mg %) a slight reduction in the alkaline reserve, from 64 4 to 59.3 an increase in thrombocytes from 200 000 to 241,000 The following laboratory values remained unchanged Lipase, acid and alkaline phosphatase, blood calcium, blood phosphorus, magne sium and the blood count.

When dogs were given (106) various amounts of 3 % NaF solution I V at 20 to 64 mg/kg (each dog received a differ ent dose) there was a 75 % decrease in excretion of t-aminohippuric acid, a simi lar decrease in excretion of creatinine, somewhat less of urea. Chloride excretion was increased 2 to 10-fold. These changes were partly reversible. There was no change in the serum urea concentration

Thus there does not appear to be any specific laboratory finding which could be considered characteristic of F intoxica tion [1]

It is likely that a disturbance of the calcium-phosphorus values in the blood prevails, namely a hypocalcemia and hy pophosphatemia. Under certain condi tions, however or perhaps at a certain phase of the illness, Ca++ levels may be found above normal.

Gettler and Ellerbrook (107) compared F levels in tissue in acute with those in chronic intoxication in rabbits. Their data are presented in Table XII.

The high F values in pituitary and adrenal glands in this study are remark able, particularly when compared with the F content of bones. Fabre et al (108) made similar observations. Such high F levels might account for the extreme weakness observed in acute intoxication by many authors.

[1] F content of gastric juices, urine and blood should be determined F-appears in the blood within few minutes after F ingestion and in urine within less than 1/2 hour

"Normal" urinary F levels range between 0.0 to 0.5 mg in 24 hours in low fluoride areas, in the blood between 0 01 and 0 1 ppm (see 96)

Roholm (105) observed an increase in diuresis[1] after toxic doses of F

c. *Pathology of Acute Intoxication*

In Animals In acute poisoning in cattle sheep, goats, swine, horses and chickens and experimental animals, focal or general congestion and submucous hemorrhages in the gastrointestinal tract are reported from NaF Na_2SiF_6 (65) and F containing superphosphate (66) and calcium phosphate (70) Hemorrhages were found in internal organs of goats (33) Pigs showed marked congestion in liver and kidneys (33) cattle poisoned by Na_2SiF_6 an increase in pericardial and intracranial fluid. Davis (109) points to acute hyperemia of viscera and to cloudy swelling and necrosis in liver and kidneys, and to a lesser extent in heart and brain of experimental animals.

In experimental exposure to airborne F (mainly HF and particular NaF) changes in the respiratory tract dominate the picture, such as irritation of mucous membranes in turbinates, trachea, conjunctivae, bronchial mucous membranes attributable to the corrosive effect of the gas Massive alveolar hemorrhages, edema, emphysema and phlegmonous bronchitis and tracheitis ensue (54) Bronchopneumonia and varying degrees of pneumonitis result, indicative of secondary infection. Machle et al (54) point to the degenerative changes in the heart and to necrosis of muscle fibers They describe atheromatous lesions in the media and cellular infiltration in intima and adventitia of blood vessels This appears to be significant in view of similar observations in chronic fluorosis in humans (110) to which so far little attention has been given.

Table VII F levels in acute and chronic experimental F intoxication (according to Gettler and Ellerbrook (107))

	Acute intoxication in rabbit of 2.49 kg after massive dose of 2.5 g NaF PPM	Chronic intoxication in rabbit of 2.80 kg after daily doses of 11 mg NaF during 4 mos. PPM
Kidney	56.5	3.1
Liver	18.3	5.2
Lung	25.3	[illegible]
Brain	56.6	4.2
Spleen	100.1	58.7
Bones	136.0	233.0
Teeth	1,490.0	2,465.0
Bile	60.3	80.0
Hypophysis.	326.0	823.0
Adrenal	413.0	—
Sex organs	6.3	3.3
Hair	68.0	110.0
Skin	15.3	10.0
Blood	14.3	9.3

At high level exposure to F in the rat Stokinger (21) observed gross and microscopic changes of the buccal mucosa. HF produced inflammatory changes in the scrotum.

In Humans The most striking changes in humans are found in the gastrointestinal mucosa. There is an acute hemorrhagic gastroenteritis with patches of hyperemia, edema and hemorrhages distributed irregularly in the stomach, duodenum, sometimes in the first portion of the jejunum, esophagus, and oral cavity The colon is usually free of pathology (105) Most authors attribute these changes to the caustic action of the F compound. Roholm (111) noted the same

[1] In one of my patients, Mrs. H. G aged 31 who experienced cephalalgia and paresthesias after ingestion of 15 mg of NaF and whose daily urinary excretion had ranged from 0.83 mg to 2.7 mg of F the 24 hour urinary volume showed an increase from 2,755 ml to 4,368 ml after the test dose

pathology following intravenous injections as frequently and as severely as following peroral intake. This led him to believe that F might be excreted into the gastrointestinal tract.

Besides this major pathology in the gastrointestinal tract, less consistent findings are passive congestion of the liver and kidneys, advanced cloudy swelling in kidneys (77) degenerative changes in the heart (107) (72) fatty degeneration of the heart muscle, liver and kidney. Gellerstedt (112) noted capillary congestion in the cerebral cortex, interlobar petechial hemorrhages and small epicardial hemorrhages (74). Roholm evidence of toxic nephritis. Kruckemeyer (113) and Lidbeck (79) emphysema at the lung margins. Maletz (74) diffuse extravasation of blood in the bronchial mucosa. Some of the pulmonary pathology especially emphysema, may conceivably have been due to inhalation of traces of the NaF powder before it was swallowed.

In the three cases reported here the only remarkable findings were those in the upper gastrointestinal tract. The other viscera appeared normal.

Faske's (93) patient showed remarkable changes in the cardiac musculature. He died 12 days after he had taken about a teaspoonful of 17.5 % HF some of which was vomited. Pinpoint to pea sized patches were present in the heart muscle, the muscle fibers were fragmented their nuclei absent wide spaces were noted between muscle fibers enclosed by sarcolemma. In contrast to Gellerstedt, Faske failed to find fatty degeneration of heart muscle cells, no exudation, hemorrhagic or granulomatous areas. The pathologic changes were confined to the muscle cells. Fatty degeneration was noted in the liver and the tubular epithelium in kidneys. There was evidence of a hypostatic pneumonia. This deviation from the findings of others might be due to the prolonged illness or perhaps to the kind of F compound in Faske's case, namely HF. Faske reported uncoagulated blood throughout the circulatory system. A strong, stringent odor of the kind never before encountered by the pathologist induced headaches in those present at the autopsy.

d. *Toxic Dose*

Like in other kinds of acute intoxication there are wide variations in the response to a given dose from individual to individual. This is illustrated by the report of Baldwin (76). Several persons had taken small doses of F. 26 gm of NaF was mistakenly added to 26 small cakes. Each cake, therefore, contained 1 gm of NaF. All vomited within 5 to 15 minutes, some had diarrhea.

A 69-year-old woman after consuming 5 cakes (*5 gm*) nausea and general weakness in 15 to 20 minutes, but no vomiting for 5 hours. Recovery took 4 weeks.

A young girl — 6 cakes (6 gm) muscular pains for several days.

Another case of Baldwin 10 gm death within 10 to 12 hours.

According to Bredemann (12) a solution of 0.25 gm of NaF produced in humans burning pains and vomiting after 1 gm, headaches after 5 gm, cardiac symptoms. Death occurred after ingestion of 10 gm.

Other fatalities resulted from swallowing 4 to 5 gm of NaF (8).

The largest dose from which a human has been known to recover was reported by Peters (88) in a woman who had taken 50 to 80 gm of NaF mixed in a 95 % suspension thicker than cream. Early vomiting ejected much of the chemical so that the patient's life could be saved.

Griebel et al (86) who investigated mass poisonings in Berlin from HF-containing apricot preserves estimated about 70 mg of F as an acutely toxic dose for an adult. This dose mixed with food caused vomiting, diarrhea and severe abdominal spasticity. Even at 50 mg of F nausea and vomiting occurred.

Gershow and Pribilla (114) reported a death within 10 minutes after swallowing 100 to 150 cc of a 13.2 % H_2SiF_6 solution.

Black and Kleiner (115) had given experimentally to persons with such incurable diseases as leukemia and cancer NaF in doses ranging from 30 to 80 mg NaF. With this minute dose they encountered nausea and vomiting. They were obliged to add Alujel to counter its effect in the upper gastrointestinal tract. The tolerance of these individuals was presumably low because of the illness with which they were afflicted.

My own data (Table IX) demonstrates that doses as low as 15 mg of NaF may do considerable harm to a small percentage of persons with a low tolerance to the drug.

For Na_2SiF_6 the lethal dose is considered to be lower than for NaF. In Dyrenfurth and Kipper's case (116) it ranged between 0.2 and 0.6 gm, in Gellerstedt's between 0.7 and 1, in Liljestrand's (91) a 3-year-old child, between 0.5 to 0.7 gm.

In contrast with the above data, some authors hold different views. Peyre (117), for instance, considers 30 gm of NaF the toxic dose in humans, for Na_2SiF_6 3 gms.

For HF and H_2SiF_6 the lethal dose is even more difficult to estimate from the available literature. Rosner's (118) patient died within 15 minutes after taking 27.5 mg of H_2SiF_6, King's (71) patient after taking 14 gm of HF. These two acids are more toxic than their sodium salts because they are more ionized.

The U. S. Department of Agriculture set 1.43 ppm as the limit of F in food and wine in 1933 (119). In 1945 the allowable limit for F from sprays used on fruit and vegetables was raised to 7 ppm (120). In self raising flour the limit in Great Britain is 3 ppm (121).

e. *Pathological Physiology*

Three modes of action appear to dominate events in acute intoxication.

Corrosive Action. The corrosive properties of F compounds are generally held responsible for the gastrointestinal symptoms. Peters (68) stored the vomitus of his patient in a glass vial in the refrigerator. It had etched the glass of the vial in which it was placed. NaF reacts with gastric HCl as follows:

NaF + HCl = NaCl + HF. *In other* words, in the presence of gastric HCl, highly corrosive HF is formed. Thus when gastric acidity is high, more extensive changes in the gastric mucosa must be expected than in hypoacidity. In the lower intestinal tract where the bowel content is alkaline, corrosive lesions are rarely found.

Another reason for absence of lesions in the bowels is the fact that F becomes more dissociated in acid medium and thus more readily absorbable into the blood stream; therefore relatively little F reaches the lower intestinal tract.

It must be assumed that urinary acidity will induce more extensive pathology in the tubular epithelium of the kidneys than an alkaline urine.

Affinity of F to Calcium and Other

After administering Na_2SiF_6 on an empty stomach of rabbits, Heydrich (81) noted less severe intoxication than when the drug was given after eating. He attributes this to an increase in gastric acidity content after meals.

Metals Early in the 20th century Loeb (122) pointed to what appears to be the major action in F toxicity F belongs to the group of chemicals which precipitate calcium in vivo. Its toxic action is similar to that of oxalic acid citric acid, oleic acid and their salts. Stuber and Lang (123) linked the delay of the coagulation time in F intoxication with its action on calcium. From the available reports, however there is no consistency in the changes in the blood coagulation. Peters (88) observed a normal prothrombin time. In his case there was no tendency to bleeding

The evidence of a disturbed Ca metabolism is supported by the extreme degree of hypocalcemia observed by Rabinowitch (92) and by Maletz (74) and by the occurrence of tetaniform convulsions As a rule they do not appear at the onset of the disease. *It seems that some time, perhaps* 2 to 3 hours, is required for the Ca deprivation. This time lag may also account for some of the inconsistencies in the blood Ca levels observed above.

Chloride loss through emesis undoubtedly contributes to depletion of ionized serum calcium. This, however plays only a minor if any part in the production of tetany Rabinowitch (92) recorded tetany without vomiting

F Actio n Enzymes There is extensive in vitro evidence that F interferes with many enzyme systems. F has a strong affinity (96) to Mg and Mn which are present in many enzymes (124) Some enzymes e.g cholinesterase are inhibited in vitro at concentrations as low as 1 5 millions. Much higher concentrations of F have been reported in tissues (See Table XII)

Fluorides block glycolysis in vivo.

Fanke (93) explained the focal fragmentation of heart muscle on the basis of F interference with the carbohydrate metabolism, specifically with the interference of glucose-6-phosphatase activity and the splitting off of phosphoric acid from the phosphorized hexose. In rabbits, however phosphorylase release activity is inhibited in the skeletal muscles, whereas that in myocardium and liver was found to remain unchanged (125)

Handler (126) produced hyperglycemia in rabbits with lethal doses of NaF This was counteracted by insulin.

Clinical data on the effect of F on glucose metabolism are sparse. Peters (88) noted glycosuria and hyperglycemia during intoxication, Fanke (93) a complete disruption of the carbohydrate metabolism with blood sugar levels ranging between 99 and 333 mg %

The interference of F with other enzymes (124) presumably accounts for certain phenomena *not understood today* F inhibition of cholinesterase, which takes place in vitro at a concentration as low as 1 5,000,000 is undoubtedly involved in the production of some of the neuromuscular manifestations observed in F intoxication.

Prissok (127) postulates a certain effect of F on the function of the thyroid gland. The available data on the thyroid gland are sparse Other endocrine glands, such as the parathyroids and pituitary (128) have also been incriminated in the mechanism of F intoxication.

f *Therapy*

There are three major steps in treating acute F intoxication

1 To remove the poison from the stomach or intestinal tract. 2. To prevent its absorption. 3 To counteract the late effect, especially that of Ca++ deprivation

Calcium therapy is considered to be the principal therapeutic measure (74 75 81

97) Given orally it will interfere with absorption of F. Intravenously it will promptly counter the hypocalcemic phenomena.

Gastric lavage should be instituted promptly and repeatedly. However plain water given as a lavage is likely to enhance the absorption of F. On the other hand, if solutions of Ca++, aluminum and magnesium salts and particularly food (milk) are added, F absorption will be retarded. Thus milk is more desirable than water as a medium for lavage.

Intravenous calcium injections should be administered continuously even when the patient appears to be improved. Death during tetaniform convulsions may appear hours after the poisoning when the patient is presumed to have recovered. A high urinary volume should be maintained by administering parenteral fluid. Intravenous glucose should be administered promptly to counter hepatic glycogen depletion. Blood transfusions are indicated to replace blood lost through hemorrhages in stomach.

Vomitus, feces and urine should be washed away to prevent external burns.

Greene's (100) case demonstrates how prompt treatment can prevent fatal outcome from intoxication with a relatively large dose:

> A 35-year-old seaman had swallowed 1.5 teaspoon (6 gm) of NaF dissolved in coffee. H as given, 2 hours later, 1 gallon of milk, the hites of six eggs, 15 of Syrup of Ipecac. He vomited violently for about 1 hour. He also eceived 2,000 cc of 5% glucose in water and 10 cc of 10% calcium gluconate, intravenously. Four days later the patient was symptom-free.

Maletz (4) recommends gastric lavage with weak lime water and magnesium sulfate in order to convert the soluble fluoride into less soluble calcium and magnesium fluoride.

2. Acute Intoxication from Airborne Fluorides

Acute intoxication by airborne F occurs mostly in industry. Since it usually involves legal repercussions, information on this type of intoxication and adequate descriptions of cases are more difficult of access than in nonindustrial poisoning. In contrast to chronic fluorosis from airborne F, acute poisoning has received much less attention.

In acute intoxication mainly two gaseous compounds are involved, HF and silico-tetrafluoride (SiF_4). Elemental fluorine gas must also be considered as a possible source because of its increasing industrial use in recent years.

As with other gaseous irritants, the primary and major area of damage caused by HF is the upper respiratory tract. The irritating or corrosive effect on the respiratory mucous membranes is evidenced by burning, stinging, rhinorrhea, conjunctival irritation, hoarseness and cough and by hemorrhages into the bronchial mucosa (12). These changes are followed by obstruction of bronchi with tenacious mucus, by dyspnea and inadequate oxygenation of blood, i.e. the clinical feature of emphysema. In severe cases, vomiting, abdominal spasm, cerebral and visual disturbances occur.

In rabbits HF is absorbed in the upper respiratory tract (129). This is in contrast with absorption of nitrogen oxide which takes place in its lower portions. This selective action of various irritating gases upon different portions of the respiratory passages is dependent on their solubility. An easily soluble gas is absorbed readily at the moist surface of the upper respiratory tract, a poorly soluble one in the lower portions of the lungs.

a. *Cases*

Roholm mentions two cases reported by Cameron in 1889 from a superphosphate factory in Dublin.

One worker was present for a moment in a chamber where crude phosphate treated with sulfuric acid was stored. He complained of dyspnea and died within a few hours. Another worker died between 8 and 10 hours after having been inside this den. His only symptoms were respiratory difficulties and a single episode of vomiting. He remained conscious until he died. At utopsy pulmonary edema and congestion was noted. The lungs contained large quantities of SiO_2, which are presumed to be deposited on the mucous membranes through decomposition of SiF_4.

Goralewski (130) described the case of a 34-year-old worker in fertilizer factory. Although he was wearing a mask, he complained of severe thirst and expectorated bloody sputum. He continued t work 4 more days during which he gradually becam worse. He died 3 months later with pulmonary abscess, bronchiolitis, pulmonary gangren and secondary anemia.

Acut pulmonary symptoms such as bronchitis, dyspnea and asthma have been reported by Evang (131) in workers exposed to dust containing F compounds.

E. R. Hayhurst, discussing a paper by Largent (132) related the case f a garage worker who had been spraying a solution of sodium fluoride without respiratory protection during the course of one day. The following day he was ill but still able t work. He became progressively worse and died on th fifth or sixth day following the exposure. N details were given

Prissok (127) reported respiratory symptoms followed by gastritis and enteritis. Upon exposure to F fumes of over 100 mg per 10 cc of air the irritation of the nasal mucous membranes was followed by rhinorrhea. This persisted for weeks and recurred frequently

Certain chlorinated fluorinated hydrocarbons used as ejecting gases in aerosol packs, although non toxic, give rise to phosgene, hydrofluoric acid, hydrochloric acid, sulphur dioxide and chlorine when they are in contact with hot surfaces in concentrations higher than the maximum permissible levels.

Among 43 cases of gas poisoning from all Swedish hospitals between 1950 and 1954 Dahlman (19) encountered 2 which were due to phosgene poisoning, resulting from Teflon heated to about 300° C Cavagena et al (133) attribute the fever encountered in this condition to particulate F compounds which are decomposition products of Teflon.

A mechanic, aged 52 years, operated with an acetylene torch upon a refrigerator which was discharging dichlorodifluoromethane (Freon-12) from a leak; he developed dyspnea, malaise and vomiting, and required hospital treatment for five days.

An gricultural worker aged 47 years, sprayed his bedroom with an aerosol fly spray containing Freon, switched on an electric heater and then went to bed. During the night he developed vomiting, diarrhea, and malaise, and died on the following day three hours after admission to hospital.

Case 6 [1] The following case f an acute toxic hepatitis which his physician and a consultant attribut d t flooride was furnished t me by Dr J G Cœur d'Alene, J R. S. aged 30, was seen on 10/7/52 because of distention, pains and abdominal distress in the right upper quadrant. Episodes of anorexia, gastric distress, loss of ppetite and general malaise during the course of 3 or 4 months had preceded this acute attack.

The patient, a water works employee, had been adding NaF to the water supply Some of the chemical necessitated sifting and removal of stones and other contaminants before it could be added to the machine. During this process clouds of F contaminated th air

The examination findings were hepatomegaly icterus, tenderness and pain in the liver region, a distinct brownish discoloration of the teeth. Roentgenographically there was inadequate concentration of dye in the gall

[1] This case is mentioned in the Journ. Amer W ter Works Assn. 49 1252 1957

bladder, no evidence of stones a slight enlargement of the duodenal loop at the head of the pancreas.

While away from work, on a low fat diet and Vitamin B, the patient improved promptly

When he resumed work on two occasions in April and again in November 1955 acute febrile recurrences were encountered with severe pain in the liver region, icterus, night sweats, fever leukocytosis, nausea, anorexia. The brown staining of teeth was noted again[1]

A consultant internist (Dr W M.) concurred in the opinion that the disease was due to F Upon voiding further exposure to F and following his transfer to a job away from the plant, the patient gradually improved and remained well. Urinary F excretion determinations further supported the diagnosis of hepatitis due to F intoxication.

Imberechts (134) reports case in greater detail

A 30-year-old male worker in an enamel factory was seized, about 1 1/2 hours after he started to work, with severe malaise, profuse perspiration, cold extremities, vomiting, and cyanosis.

The pulse became imperceptibl temperature dropped to 35 4 C. The tongue showed hemorrhagic areas. Clonic spasms of the abdominal muscles were accompanied by severe vomiting.

Investigation of the patient meals ruled out intoxication from this source. Being in charge of a kiln, he had been working in dust containing fluoride.

Following emergency treatment with analeptics and intravenous saline solution, the following laboratory data were recorded Urine Specific Gravity 1.026, PH 6.2, NaCl 7.57 ppm, NaF 12 8 ppm. Ammonium Salts 1.08 gm. Bilirubin + + + + The blood showed RBC 2,700,000 WBC 7,500, hemoglobin 40 % neutropenia and lymphocytosis.

After 48 hours, the acut symptoms subsided, but the patient continued to suffer from marked myasthenia and evidenc of myocarditis which required bed rest for about 3 months. During this time he eceived several blood transfusions. He was totally disabled for 9 months.

After resuming work in the same factory against the physician s advice, he was taken by another seizure 48 hours later At this time the urine showed CaF_2 of 9 ppm (4.32 mg F) one week later 5 ppm (1.67 mg of F) After this episode the patient was disabled for 3 months, following which he changed his occupation and recovered.

[1] Pepperkorn und Kahling (135) reported presence of brownish film removable by scraping in industrial fluorosis This should not be confused with mottling of teeth

b. *Toxic Dose of Inhaled F*

The duration and magnitude of exposure determines the outcome in air-borne F intoxication. Machle et al (54) described the effect of short term exposures to HF in two individuals

At a level of 0.1 mg/liter of F as HF they experienced smarting of the skin, conjunctival and respiratory irritation in less than 1 minute. The taste of the gas was very pronounced.

At 0.05 mg/l only conjunctival and nasal irritation, tickling and discomfort of the air passages with each inspiration. The taste was still definite.

At 0.026 mg/l some discomfort but the atmosphere could be tolerated for several minutes. There was no tendency toward habituation to this concentration by repeated exposures.

Largent (137) maintains that exposures of humans to HF up to 10 ppm causes no material damage. The symptoms encountered at much higher F levels extreme burning, pain in eyes, nose, mouth, pharynx and upper chest, rhinorrhea, lacrimation, tingling and burning of the skin, gasping, coughing and laryngospasm.

These short term exposures do not reflect conditions in factories or in F contaminated areas where exposures are much more protracted.

Rabinowitch (92) states that as little as 10 ppm of F in air produces ulceration in the respiratory tract and bronchopneumonia. He also quotes the maximum allowable concentration for prolonged exposure as 2.5 mg per cc m or approximately 3 ppm of HF

While visiting an aluminum plant he observed on himself marked lacrimation, sensation of suffocation and cough which continued about 1/2 hour after he had left the contaminated atmosphere. None of these symptoms, however was noticed by any of the workers with whom he had contact.

c. *Treatment*

Treatment of acute intoxication from air contaminant F should be directed toward relieving cough and expectoration, to countering nasal and sinus irritation preventing secondary infection and development of pulmonary emphysema. For prevention of infection, extensive use of antibiotics is indicated to counter the development of pulmonary emphysema, steroids in large doses should be given. Bronchial evacuation by epinephrine-like inhalants combined with bronchial detergents is indicated.

3 Local Action of Fluorides

HF and SiF_4 and the more soluble F compounds exert a corrosive action on living membranes. Brief exposure is known to cause redness and prolonged burning sensation. Longer exposures with more concentrated solutions produce yellowish excoriations which later develop into painful ulcers (136) Such lesions are rarely fatal although some cases have been described.

Stokinger (21) pointed to the difference of the local effect of F_2 gas and that of HF upon the skin of rabbits

The flash burn of pure F gas is identical with second degree thermal burns. On the other hand, HF produces no immediate reaction within a few days, erythema of the epidermis with small dark areas of liquefaction necrosis appears. Healing occurs more slowly than with F_2 burns and is characterized by multiple eschar formation in the necrotic areas. Stokinger's experimental lesions from HF did not heal for 27 days, whereas burns from elemental F_2 gas were completely healed within two weeks.

HF burns encountered in industry differ according to whether the acid is concentrated, diluted or in vapor From concentrated acid the burn described above is extremely painful. The pain is immediate and resistant even to morphine. If treatment is not prompt, necrosis proceeds in depth (138) If the acid is less concentrated, only slight erythema appears at first, the burn a few days later

Since HF is odorless, persons exposed to it do not become aware of the danger.

a. *Treatment of F Burns*

The treatment of hydrofluoric acid corrosions of the skin has recently been summarized by Buffet (138)

Right on the spot at the place of work the wound is immediately cleansed under running water for at least 10 minutes.

In the emergency room of the factory cleaning is to be continued under running water or with sodium bicarbonate solution. In less severe cases the wound may be dressed with 7 % magnesium oxide paste.

If there is tissue necrosis, a circumferential injection of 10 % calcium gluconate should be made. About 1 to 30 cc, according to the size of the wound area, should be injected at several sites. The best test to determine whether or not sufficient calcium has been injected is the disappearance of pain which takes place within 10 to 15 minutes. The calcium injection is surprisingly painless It may or may not be preceded by procaine infiltration.

Thereafter blebs should be opened, covered with magnesium paste and a sterile dressing The speed of administering this treatment may determine its outcome

Where there is only simple irritation of the skin with erythema and relatively little pain, topical application of a magnesium oxide paste of the following formula is useful

	Grams
MgO	20.0
$MgSO$	100.0
Glycerine	100 0
Gum Arabic	2.0
H_2O	150 0

In the eye F burns require prolonged and copious irrigation with tepid tap water (138) This is to be followed by 3 % boric acid solution. Subsequent treatment consists of application of pantocaine for relief of pain, instillation of mydriatics and of removal of any necrotic tissue in the cornea. Ointments should not be applied, either to skin or to the eye.

Buffet described the following case

A worker was splashed by a few drops of HF on his left arm. His arms were raised at the time; thus the acid ran down th gloves on the left forearm. The lesions were immediately washed off with tap water for 10 minutes. The pain was very severe as though he had touched some steam. There was a 3rd degree burn 13 cm by 2 cm. A grayish-white center in an area of necrosis was noted, but no blebs.

After another bath with tap water for 10 more minutes, 20 cc of calcium gluconate was injected around the burn, followed by an application of magnesium oxide paste, and sterile management. Within 15 minutes the pain disappeared, the inflammation subsided after 7 days; a white scar remained for 2 more weeks

An ingenious method for the treatment of HF burns was recently described by Wild (139) He uses the enzyme Hyaluronidase in order to retain both calcium and procaine at the site of damage.

His *Solution I* contains an ampule of Hyaluronidase with 350 International units dissolved in 30 ml of a 2 % procaine solution.

Instead of this freshly prepared solution, there is a suitable stable solution of hyaluronidase in Lidocainum 2 %

Solution II contains 4 % procaine with 20 % calcium gluconolactobionate (Sandoz)

Wild injects solution I immediately where pain occurs. This is followed by injection of solution II in a proportion of 1 2. The total amount depends on the degree of pain and the extent of the affected area. As a rule, 2 or 3 injections are made in 24 hours.

Wild relates the case of a workman who touched with the tip of his left ring finger a mixture of 70 % HF and HCL. Workmen called this solution mistakenly "hydrochloric acid" At first ther was no pain, nor lesion on the finger After 90 minutes most intense pains occurred, involving the whole forearm. The physician, who considered this as a "hydrochloric acid" burn, did not realize that the mixture contained HF Thus several hours passed until he began effectiv treatment.

In another case, one drop of HF touched the tip of a fingernail. In spit of immediat careful washing with ammonium solution to counteract the "acidity" (the alkaline solution is useless because there is little or no acidity effect of HF) the area showed a small, slightly yellow-white discoloration Seven hours later severe pains occurred lasting for 30 hours. Interestingly the nail itself did not suffer but ther was a pea sized necrotic lesion below the nail.

b. *Local Allergic Reactions*

In discussing local F effect, allergic reactions due to applying F compounds to teeth should be mentioned.

During own studies on fluoride intoxication from drinking water (96) data have been accumulated indicating that fluoride may cause allergic reactions (9) even when taken in minute doses.

A 62-year-old femal (Mrs. F F H.) (9) following use of stannous fluoride toothpaste (approximately 1 mg of F) showed edematous pruritic lesions with a 12 % eosinophilia on the oral mucosa which was followed by ulceration. This was associated with paresthesias in the arms.

Hyperemia, edema and ulcerations were reproduced by application of a cotton swab soaked in a 1 % solution of sodium fluoride, pplied t a test site in the mucosa of the lower lip. A control test with saline solution was negative. The patient had not been aware which swab contained F On a subsequent blind test with the fluoride toothpaste the patient developed in addition to th oral lesions a severe colitis.

Douglas (139) reported 133 cases with stomatitis caused by fluoride containing toothpastes and tooth powders. The lesions were reproduced in 32 cases, in some as often as 6 times.

Spencer (140) of Bloomington, Ind where the original experiments with stannous fluoride toothpaste were made, observed three cases of stomatitis following the application of stannous fluoride which were proven by blind and patch tests.

A 3-year-old allergic girl developed a severe case of stomatitis and cheilitis following use of stannous toothpaste. It was followed by marked edema of the oral mucosa, salivation and temperature ranging up to 101.2 (Dr C.D.M., Memphis, Tenn.) lasting for 5 days. The condition necessitated hospitalization. Edematous and membranaceous lesions involved the buccal mucosa and both margins of the tongue. There was marked salivation. Patch tests incriminated the F in the toothpaste.

Fregert and Moller (142) reported a case of dermatitis from eye drops containing a fluoride alkyl phosphate. Two other similar compounds exhibited crossreactions on epicutaneous testing

DISCUSSION

Judging from reports of acute mass F^- intoxication, some of which have taken place recently, the disease appears to be more frequent than the relatively few reports in the literature indicate. The institution of poisoning centers by health departments may lead to a more adequate appraisal of its incidence. Nevertheless, many cases of F^- intoxication are bound to remain undiagnosed. The lack of taste and smell of the common F^- compounds, the unavailability in general hospitals of easy and prompt methods of F^- analysis, and the lack of familiarity with the disease among practicing physicians has been and will remain a handicap in establishing the diagnosis.

In industry the incidence of the disease is even more difficult to evaluate. Industrial cases usually lead to litigation; therefore, data are rarely available to investigators outside of the plant.

The major and sometimes the only feature of acute F^- poisoning are the manifestations attributable to the corrosive changes in the upper gastrointestinal tract. In one of McNally's (75) cases even these symptoms were absent and no complaints other than weakness occurred during the 3 1/2 hours between the ingestion of an unknown quantity of NaF and death. Thus death may occur without any striking manifestations.

This lack of diagnostic features is borne out by the sparsity or indeed the absence of conspicuous pathology at autopsy. Raestrup (97) pointed to the bright red appearance of the skin and the hemorrhagic stomach content; others, to the finding of uncoagulated blood at autopsy. Neither feature, however, is consistent enough to be pathognomonic. History, high F^- level in gastric content, in urine and in tissues and perhaps low calcium values in the blood are diagnostically significant.

The full mechanism of the action of F^- on the system is not clear at present. The most widely accepted theory is the assumption that the calcium precipitating action of F^- is the major determinant of its effect, particularly in the central nervous system. In addition, the strong affinity of F^- to most metals present in the system must be reckoned with. Very little is known to what extent this interferes with the activity of many enzymes in vivo.

In contrast to an enormous amount of literature pertaining to biochemical and dental aspects of F^-, the clinical effect of F^- in humans has received very little attention. In view of the marked toxicity of certain F^- compounds and its increasingly widespread use in industry, greater dissemination of knowledge of clinical data on this subject among physicians and lay persons alike is indicated.

SUMMARY

1. Acute intoxication with fluoride compounds through accidental exposure, homicides and suicides, is not uncommon. Epidemics of rather serious proportions have been caused by mistaking fluoride compounds for baking soda, flour, sugar, etc.

2. There are *wide variations in response* to fluoride intake, depending on such factors as the particular fluoride compound involved, the animal species and on various biological conditions in humans. Inorganic fluorides, especially NaF and Na_2SiF_6, are more frequently involved in acute intoxication than organic compounds. HF, H_2SiF_6 and particulate NaF are mostly responsible for acute intoxication from air contamination.

3. Three autopsied cases of intoxication with NaF are reported, namely two homicides and one accidental case.

4. Clinical, autopsy and laboratory findings of acute fluoride intoxication are sparse and inconsistent. Hemorrhagic gastritis, tetaniform convulsions, hypocalcemia and hyperfluoruria are the principal clinical findings. There are no pathognomonic changes at autopsy. *Corrosive lesions in the upper gastro*-intestinal tract, congestion and hyperemia in other vital organs are usually encountered. Changes suggestive of corrosion have been reported in the respiratory tract in intoxication from air borne fluoride.

5. Therapy should be directed toward removal of the poison from the stomach and intestinal tract, toward retarding its absorption into the blood stream, toward countering calcium deprivation in the blood.

6. A description of the topical effect of fluoride and of allergic reactions is presented.

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ADDENDUM

Thiele and Wild (143) reported recently on 179 patients who were treated by Wild's method, supplemented with local application of hydrocortisone ointment. In 111 patients thus treated the average duration of disability was 14.12 days. In 68 patients treated without Hyalurinidase the average duration of the disability was 20.4 days.

Many of these patients had splash burns from a mixture of fluoride and aluminum oxide molten at 950° Celsius. There was an immediate effect from the burn on the affected site. The fluoride necrosis followed a few days later after the fluoride ion had penetrated into the subcutaneous tissue.

Dieffenbacher and Thompson (144) reported 2 cases of extensive HF burns. One terminated fatally.

A worker in an oil refinery was burned by 10 % anhydrous HF in propane in contact with the skin for 30—35 seconds. Face, both ears and the anterior and lateral aspects of the neck were involved. Bathing in a tank with soda bicarb in travenous and intradermal injections with calcium gluconate and supportive treatment with oxygen were of no avail. He died within 2 1/4 hours.

The autopsy revealed subserous hemorrhages of the pleura hemorrhages of the posterior portion of the intraventricular septum injection and hemorrhages in tracheal and bronchial membranes. The bronchi contained bloody mucus. Both lungs showed edema and hemorrhages. The kidneys exhibited numerous subepithelial hemorrhag the brain marked hyperemia

The second patient was struck by an oil HF mixture blown through a U tube toward his chest. Some of the mixture found its way underneath the protective neck piece and hood and came in contact with his skin. HF vapors inside his protective helmet caused respiratory difficulties.

The chest showed moist rales. The heart rate was irregular and rapid, blood pressure 220/180.

He was treated extensively with calcium gluconate intravenously and intradermally later with ointment containing

Fig. 5. Harmless appearing splash burn from aluminum-fluoride mixture molten at 950° C. During the first 2 days hardly noticeable and painless lesions of pea size. Immediate treatment. No disability (Courtesy Dr. H. Wild, Basel.)